대한진단검사의학회지 제29권 제3호 2009
Korean J Lab Med 2009;29:231-7
DOI 10.3343/kjlm.2009.29.3.231

DNA 정제 없이 전혈을 사용한 빠른 ABO 유전형 검사

이성호[1] · 박 건[2] · 양영근[3] · 이승관[4] · 김성욱[4]

루미아이 제네틱스[1], 조선대학교 의과대학 진단검사의학교실[2], 바이오퀘스트[3], 고려대학교 임상병리학과[4]

Rapid ABO Genotyping Using Whole Blood without DNA Purification

Sung Ho Lee, Ph.D.[1], Geon Park, M.D.[2], Young Geun Yang, Ph.D.[3], Seung Gwan Lee, Ph.D.[4], and Suhng Wook Kim, Ph.D.[4]

R&D Center[1], Lumieye Genetics Co., Ltd., Seoul; Department of Laboratory Medicine[2], Chosun University College of Medicine, Gwangju; BioQuest[3], Inc., Seoul; Department of Clinical Laboratory Science[4], College of Health Sciences, Korea University, Seoul, Korea

Background : ABO genotyping is commonly used in cases of an ABO discrepancy between cell typing and serum typing, as well as in forensic practice for personal identification and paternity testing. We evaluated ABO genotyping via multiplex allele-specific PCR (ASPCR) amplification using whole blood samples without DNA purification.

Methods : A four-reaction multiplex ASPCR genotyping assay was designed to detect specific nucleotide sequence differences between the six ABO alleles *A101, A102, B101, O01, O02*, and *cis-AB01*. The ABO genotypes of 127 randomly chosen samples were determined using the new multiplex ASPCR method.

Results : The genotypes of the 127 samples were found to be *A101/A102* (n=1), *A102/A102* (n=9), *A101/O01* (n=3), *A102/O01* (n=12), *A102/O02* (n=14), *B101/B101* (n=5), *B101/O01* (n=18), *B101/O02* (n=15), *O01/O01* (n=14), *O02/O02* (n=8), *O01/O02* (n=14) and *A102/B101* (n=14), from which phenotypes were calculated to be A (n=39), B (n=38), O (n=36) and AB (n=14). The multiplex ASPCR assay results were compared with the serologically determined blood group phenotypes and genotypes determined by DNA sequencing, and there were no discrepancies.

Conclusions : This convenient multiplex ASPCR assay, performed using whole blood samples, provides a supplement to routine serological ABO typing and might also be useful in other genotyping applications. *(Korean J Lab Med 2009;29:231-7)*

Key Words : *ABO genotyping; Whole blood; Allele-specific polymerase chain reaction*

INTRODUCTION

The ABO blood group is one of the most important blood group antigens that must be correctly identified prior to transfusion and transplantation procedures. Serological ABO typing is performed using the anti-A and anti-B antisera of polyclonal or monoclonal origin, which can distinguish the four phenotypes (A, B, AB, and O). Since the first delineation of the molecular basis of the ABO blood group by Yamamoto et al. [1, 2], it has become possible to determine the ABO genotypes using molecular biology methods without the need for family investigations. ABO genotyping is commonly used in cases of an ABO discrepancy between cell typing and serum typing, as well as in forensic practices

Received : July 21, 2008 Manuscript No : KJLM2155
Revision received : April 23, 2009
Accepted : April 23, 2009
Corresponding author : Suhng Wook Kim, Ph.D.
Department of Clinical Laboratory Science, College of Health Sciences, Korea University, San-1 Jeongreung-dong, Seongbuk-gu, Seoul 136-703, Korea
Tel : +82-2-940-2811, Fax : +82-2-917-2388
E-mail : swkimkorea@korea.ac.kr

for personal identification and paternity testing.

There are several polymerase chain reaction (PCR) based methods used to identify the ABO genotypes, such as PCR followed by restriction digestion [3, 4], denaturing gradient gel electrophoresis [5], single-strand conformation polymorphism [6], and allele-specific PCR [7, 8]. Although PCR is technically simple, it is labor-intensive and time-consuming. The rate-limiting step is usually the extraction of DNA from whole blood samples. A rapid and simple method for ABO genotyping is needed due to the importance of speed in practical ABO genotyping.

Multiplex allele-specific PCR (ASPCR) technology is able to meet these criteria for its advantages in cost, reaction time, and simplicity of handling. Furthermore, the technique has been successfully used for genotyping in various human mutations [9-11]. Therefore, multiplex ASPCR represents an alternative to current standard ABO genotyping protocols as PCR followed by restriction digestion or sequencing. However, to take full advantage of the potentiality of multiplex ASPCR and to accelerate the entire analytical procedure, a simple, rapid and cost-saving method to prepare DNA from a high number of clinical blood samples is also needed.

This paper reports a rapid and simple method for ABO genotyping via multiplex ASPCR amplification using whole blood as the DNA source.

MATERIALS AND METHODS

1. Materials

A total of 127 peripheral blood samples, collected in EDTA tubes, were obtained from unrelated blood donors who provided informed consent to participate in this study. A *cis*-AB blood sample whose genotype was determined by DNA sequencing was obtained from the Chosun University Hospital. The protocol was approved by the Institutional Review Board of Lumieye Genetics. The ABO phenotype was determined by standard serological method and the ABO genotype was determined by DNA sequencing.

Determination of ABO phenotype by serological methods was done using commercially available murine monoclonal anti-A and anti-B antibodies (Bioscot, Livingston, UK). Reverse typing was done by the tube method using A_1 and B cells. For the 127 samples, the numbers of serologically determined blood groups were: A (n=39), B (n=38), O (n=36), and AB (n=14).

For DNA sequencing, a 2,051 bp fragment spanning exons 6 and 7, and intron 6 was amplified using the primer pair ABOe6F1 (5′-TGCAGTAGGAAGGATGTGCTCG TG-3′) and ABOi7R4 (5′-ACAACAGGACGGACAAAGGAAACAG-3′) as described previously [12]. PCR products were electrophoresed in agarose gel and purified using Qiaquik gel extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The purified PCR products were sequenced using a Big Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 3100 Genetic Analyser (Applied Biosystems) according to the manufacturer's instructions. Based on DNA sequencing, the genotypes of the 127 samples were: *A101/A102* (n=1), *A102/A102* (n=9), *A101/O01* (n=3), *A102/O01* (n=12), *A102/O02* (n=14), *B101/B101* (n=5), *B101/O01* (n=18), *B101/O02* (n=15), *O01/O01* (n=14), *O02/O02* (n=8), *O01/O02* (n=14) and *A102/B101* (n=14).

2. Methods

1) Primer design

The design of the oligonucleotide primers was based on the nucleotide sequence of the ABO gene [2]. In order to meet the requirement that nucleotide substitutions are clearly related to specific phenotypes, this study focused on the nucleotide positions 261, 297, 467, and 803, so as to discriminate between the *A101, A102, B101, O01, O02,* and *cis-AB01* alleles, as shown in Fig 1. Table 1 shows the oligonucleotide primer sequences, their combinations, amplification product lengths, and allele specificities. The 3′ base of each primer (except int6) was designed to correspond to the nucleotides at positions 261, 297, 467, and 803, which define the polymorphisms. Primer int6 is a common primer and its sequence is located in intron 6. Allele specificity was further enhanced by introducing additional deliberate mismatches to the oligonucleotide primer sequences at position -2

from the 3'-terminus.

2) PCR reactions

PCR was carried out in a 20 μL mixture containing 1× AnyDirect solution (Bioquest, Seoul, Korea), 1.5 μL of EDTA-treated whole blood, 0.5 μM of each allele-specific primer, 1 pg of bacteriophage Lamda genomic DNA, 0.2 μM of each control Lamda primer, 0.2 mM of each dNTP, 4.5 mM $MgCl_2$, and 2 unit of *Taq* DNA polymerase (Bioquest, Seoul, Korea).

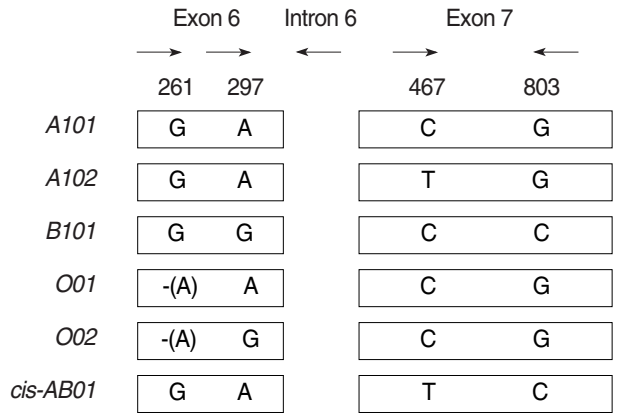

Fig. 1. Primer positions for ABO genotyping. The key polymorphisms are indicated at positions, 261, 297, 467, and 803. Alleles *O01* and *O02* have a G deletion at nucleotide position 261 (the next nucleotide is shown in parentheses). Nucleotides at these positions were detected in PCR reactions using the appropriate primers as shown with arrows (given in Table 1).

PCR was performed in a thermal cycler (Clemens, Germany) preheated to 95℃. The cycling conditions were: initial denaturation for 5 min at 95℃, followed by 35 cycles of 40s at 95℃, 40s at 60℃, 40s at 72℃, and an additional 5 min at 72℃ for final elongation. After the reaction, the PCR mixture (10 μL) was electrophoresed on a 2% agarose gel and stained with ethidium bromide.

An internal control template and primers were used to confirm the reliability and reproducibility of each assay. In each PCR reaction, control bacteriophage Lamda genomic DNA template and control primers (forward, 5′-gat gag ttc gtg tcc gta caa ctg g-3′; reverse, 5′-ggt tat cga aat cag cca ca-3′) were used to amplify a 500 bp PCR fragment, which corresponded to nucleotides 7,131-7,630 of the Lambda genome and served as a positive amplification control [13].

RESULTS

Our multiplex ASPCR assay contains four independent PCR reactions (Table 1). In the first reaction, the 261G-int6 primer pair was selected to amplify the 205 bp fragment in order to detect the *A101, A102, B101,* and *cis-AB01* alleles, and the 467C-803G primer pair was selected to amplify the 381 bp fragment to detect the *A101, O01,* and *O02* alleles. In the second reaction, the 297A-int6 primer pair

Table 1. Primers used for ABO genotyping

PCR reaction	Primer pair	Fragment size (bp)	Allele specificity
1	261G: 5′-GCAGTAGGAAGGATGTCCTCGTGtTG-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	205	*A101, A102, B101, cis-AB01*
	467C: 5′-CCACTACTATGTCTTCACCGACCAtCC-3′ 803G: 5′-CACCGACCCCCCGAAGAtCC-3′	381	*A101, O01, O02*
2	297A: 5′-CCATTGTCTGGGAGGGCcCA-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	164	*A101, A102, O01, cis-AB01*
	467C: 5′-CCACTACTATGTCTTCACCGACCAtCC-3′ 803C: 5′-CACCGACCCCCCGAAGAtCG-3′	381	*B101*
3	261A: 5′-GCAGTAGGAAGGATGTCCTCGTGtTA-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	205	*O01, O02*
	467T: 5′-CCACTACTATGTCTTCACCGACCAtCT-3′ 803G: 5′-CACCGACCCCCCGAAGAtCC-3′	381	*A102*
4	297G: 5′-CCATTGTCTGGGAGGGCcCG-3′ int6: 5′-AGACCTCAATGTCCACAGTCACTCG-3′	164	*B101, O02*
	467T: 5′-CCACTACTATGTCTTCACCGACCAtCT-3′ 803C: 5′-CACCGACCCCCCGAAGAtCG-3′	381	*cis-AB01*

The primers are named based on the position of their 3' end relative to the cDNA sequence of *A101* allele. The nucleotides not complementary with the target template are in lower case.

was selected to amplify the 164 bp fragment to detect the *A101, A102, O01,* and *cis-AB01* alleles, and the 467C-803C primer pair was selected to amplify the 381 bp fragment to detect the *B101* allele. In the third reaction, the 261A-int6 primer pair was selected to amplify the 205 bp product to detect the *O01* and *O02* alleles, and the 467T-803G primer pair was selected to amplify the 381 bp product to detect the *A102* allele. In the fourth reaction, the 297G-int6 primer pair was selected to amplify the 164 bp product to detect the *B101* and *O02* alleles, and the 467T-803C primer pair was selected to amplify the 381 bp product to detect the *cis-AB01* allele.

Random mating, with the six different alleles at the ABO locus, can result in 21 different genotype combinations, which are all detectable using the developed method. However, only 12 different genotype combinations were detected from the 127 randomly chosen samples (Fig. 2). The *A101/A101*, *A101/O02* and *A101/B101* genotypes and the *cis-AB01* containing genotypes could not be detected. This observation is not unexpected given the frequency of these alleles and the size of the study group. In order to test the specificity of the *cis-AB01* allele specific primer pair (467T-803C), we include a *cis*-AB blood sample in our test, and the genotype was identified as *cis-AB01/O02*. Fig. 2 shows the agarose gel electrophoresis patterns for the 13 genotypes.

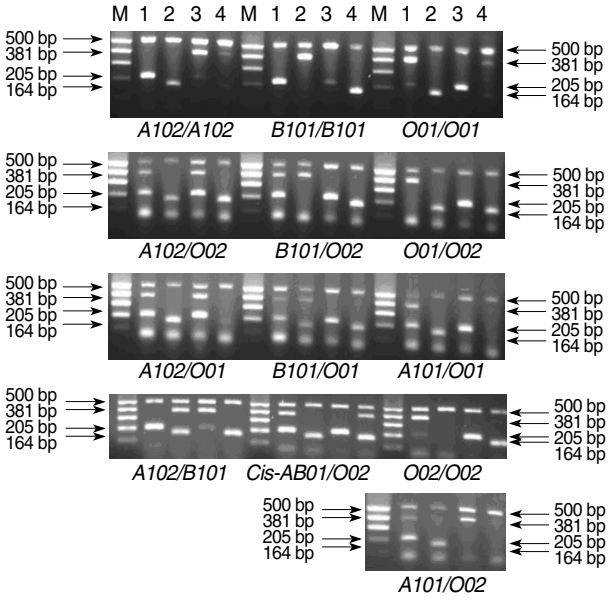

Fig. 2. Electropherogram of selected ABO genotypes. The gel photograph showing the size difference of the amplified products was obtained from the samples with 13 independent genotypes. The internal control produces a distinct 500 bp band in all four reactions. Lane M, size marker (100-500 bp ladder); lane 1, PCR reaction 1; lane 2, PCR reaction 2; lane 3, PCR reaction 3; lane 4, PCR reaction 4.

With our multiplex ASPCR assay, the genotypes of the 127 samples were found to be *A101/A102* (n=1), *A102/A102* (n=9), *A101/O01* (n=3), *A102/O01* (n=12), *A102/O02* (n=14), *B101/B101* (n=5), *B101/O01* (n=18), *B101/O02* (n=15), *O01/O01* (n=14), *O02/O02* (n=8), *O01/O02* (n=14), and *A102/B101* (n=14), from which phenotypes were calculated to be A (n=39), B (n=38), O (n=36), and AB (n=14). The multiplex ASPCR assay results were compared with the serologically determined blood group phenotypes and genotypes determined by DNA sequencing, and there were no discrepancies. These results demonstrated that the ASPCR method described here is reliable and accurate.

DISCUSSION

PCRs using allele-specific primers is a powerful tool for discriminating between alleles arising from single base substitutions or deletions without the necessity of post-amplification restriction endonuclease digestion or hybridization. The allele discrimination in ASPCR is based on the inability of Taq DNA polymerase, which lacks the 3′-exonucleolytic proofreading activity, to extend a primer when the 3' nucleotide is not complementary to a DNA template.

ASPCR with unmodified, mutation-specific primers is commonly used to detect mutations. However, such primers may yield false-positive amplifications, even under optimized conditions. Such false-positive results are unacceptable during the testing of clinical samples. Accurate detection of mutations using allele-specific DNA primers requires an investment in primer design, optimization time, and reagents. Furthermore, the ability to develop reliable multiplex reactions is difficult because of primer/template design considerations and the need to balance PCR conditions with specificity for all targets. As a result, development of an approach that amplifies all alleles accurately would enhance the confidence in ASPCR as a reliable technique. Another

significant drawback of the ASPCR assay is that mutation-specific primers introduce the mutation of interest into the amplified product. Thus, it is not possible to confirm a positive result by direct DNA sequencing of the amplicon.

A multiplex ASPCR assay containing four independent PCR reactions was developed for direct genotyping of the *A101, A102, B101, O01, O02*, and *cis-AB01* alleles (Table 1). Proper design of the primer DNA sequences is important for efficient detection in ASPCR because unexpected primer extension with mismatch-forming DNA primers can occur when PCR is carried out under unsuitable reaction conditions with regard to the amplification cycle, reaction time and temperature. An extra destabilizing mismatch was reported to increase the specificity of ASPCR [14]. Therefore, allele-specific primers were designed to make the 3' end nucleotide specific to the nucleotides at positions 261, 297, 467, and 803, which discriminate between the *A101, A102, B101, O01, O02,* and *cis-AB01* alleles (Fig. 1). In addition, the 2nd nucleotide from the 3' end was mismatched with the template DNA.

Using the multiplex ASPCR assay, we could accurately genotype 127 blood samples. However, we could not detect genotypes *A101/A101, A101/O02,* and *A101/B101* and the *cis-AB01* containing genotypes due to the low frequency of these alleles. Song et al. genotyped 222 samples and reported the ABO genotype frequencies of *A101/A101, A101/O02*, and *A101/B101* to be 0.00%, 1.35%, and 0.45%, respectively [15]. Also, the cis-AB blood group is rare and the overall frequency of this blood group was determined to be 0.0354% among southwestern Korean blood donors [16].

The multiplex ASPCR assay was performed directly using whole blood samples without DNA purification. This was successfully carried out using the AnyDirect solution, which has been used to amplify target DNA fragments directly from whole blood [17], plant tissue [18] and human feces [19] without the need for DNA extraction. AnyDirect solution is a new PCR reaction buffer that conserves the enzyme activity of DNA polymerases for effective use in direct PCR from whole blood, which contains PCR inhibitors [17]. AnyDirect solution is primarily composed of zwitterionic buffer and PCR amplification facilitators that suppress PCR inhibitors in whole blood. In our assay, a small aliquot (1.5 μL) of whole blood was added directly to a PCR mix containing AnyDirect solution and then subjected to PCR. The initial PCR step, heating to 95°C, was sufficient to lyse blood cells and liberate their genomic DNA contents; therefore, the target DNA fragments were efficiently amplified (Fig. 2).

PCR performed directly on whole blood is a more rapid and simple approach because DNA purification is both time-consuming and labor intensive. Furthermore, direct PCR performed on whole blood solves the problems associated with DNA purification, including the loss of trace samples, exposure to toxic reagents, and the difficulty in automation for large-scale diagnosis. Another significant advantage of performing direct PCR on whole blood is the hot-start effect because an initial heating step is needed to release the genomic DNA from blood cells. Hot start PCR often eliminates the nonspecific reactions in multiplex PCR, particularly the production of primer dimers caused by primer annealing at low temperatures before the commencement of thermocycling [20].

Several direct PCR protocols that do not require DNA isolation have been developed. The simple pretreatment of whole blood with heat [21], microwave [22], or formamide [23] has been attempted for direct PCR amplification of the target DNA. Unfortunately, these methods failed to generate successful results, and significant variations were observed in the data obtained. Recently, Ampdirect solution (Shimadzu, Kyoto, Japan) was developed and used to amplify the target DNA from human whole blood [24]. However, this solution was ineffective when used with chemically modified *Taq* DNA polymerases [24]. In contrast, AnyDirect solution was used successfully for direct amplification from low copy number DNA in whole blood with chemically modified hot-start *Taq* DNA polymerase [17].

Specificity and sensitivity are particularly crucial for diagnostic applications when the target is in poor quality, as occurs in whole blood. The specificity and sensitivity of direct PCR using AnyDirect solution was previously compared using purified genomic DNA and whole blood as templates [17]. The specificity of direct PCR using AnyDirect solution was demonstrated by detecting variably sized tar-

get DNA sequences of the p53 gene from whole blood without isolation of the DNA. Amplification of fragments of the p53 gene that ranged from 146 bp to 1,702 bp from purified human genomic DNA using conventional PCR reaction buffer produced weak and smeared bands or nonspecific bands. However, a single PCR product of the expected size was amplified from whole blood samples in the presence of AnyDirect solution [17].

The sensitivity of direct PCR using AnyDirect solution was determined by testing the decreasing copy number of a plasmid containing the HIV-1 genome. Using AnyDirect solution, low copy number DNA targets were effectively amplified from whole blood, and the sensitivity of detection was determined by obtaining a positive amplification signal using 10 copies of HIV-1 plasmid in 1 μL whole blood as the PCR template [17]. The specificity and sensitivity of our multiplex ASPCR assay was also confirmed by simultaneous detection of all three PCR products from 1.5 μL whole blood samples in a single reaction tube (Fig. 2).

In conclusion, this new multiplex ASPCR assay performed on whole blood is a rapid, simple, and convenient test. In addition, this fast and accurate technique offers a supplement to routine serological ABO typing results, and might also be useful in forensic applications.

요 약

배경 : ABO 유전형 검사는 혈청검사와 혈구검사의 불일치가 있는 경우와 개인확인 및 친자확인 등 법의학에서 자주 사용된다. 저자들은 DNA를 정제하지 않고 전혈을 직접 사용한 multiplex allele-specific PCR (ASPCR)을 ABO 유전형 검사법에 적용하고 그 유용성을 검토하고자 하였다.

방법 : 여섯 개의 ABO 대립유전자인 *A101*, *A102*, *B101*, *O01*, *O02*, *cis-AB01* 사이의 특이적인 염기서열 차이를 검출할 수 있는 4개의 반응을 사용하는 multiplex ASPCR 유전형 검사법을 고안하였다. 이 새로운 multiplex ASPCR 방법을 사용하여 127개의 무작위적으로 선택된 시료의 ABO 유전형을 결정하였다.

결과 : 127검체의 유전형은 *A101/A102* (n=1), *A102/A102* (n=9), *A101/O01* (n=3), *A102/O01* (n=12), *A102/O02* (n=14), *B101/B101* (n=5), *B101/O01* (n=18), *B101/O02* (n=15), *O01/O01* (n=14), *O02/O02* (n=8), *O01/O02* (n=14), *A102/B101* (n=14)였으며 이로부터 계산된 혈액형 표현형은 A (n=39), B (n=38), O (n=36), AB (n=14)였다. 혈청학적으로 결정된 혈액형 표현형과 염기서열분석을 통해 결정된 유전형을 multiplex ASPCR 검사법을 사용한 결과와 비교하였을 때 모두 일치하였다.

결론 : 전혈을 사용하여 수행되는 편리한 multiplex ASPCR 방법은 일상적인 혈청학적 ABO 혈액형 검사의 보조수단을 제공하고 유전형검사가 필요한 분야에도 유용하게 사용될 수 있을 것으로 여겨졌다.

REFERENCES

1. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature 1990;345:229-33.
2. Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S. Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc alpha 1→2Gal alpha 1→3GalNAc transferase (histo-blood group A transferase) mRNA. J Biol Chem 1990;265:1146-51.
3. Chang JG, Lee LS, Chen PH, Liu TC, Lin SF, Lee JC. Rapid genotyping of ABO blood group. Blood 1992;79:2176-7.
4. Lee JC and Chang JG. ABO genotyping by polymerase chain reaction. J Forensic Sci 1992;37:1269-75.
5. Johnson PH and Hopkinson DA. Detection of ABO blood group polymorphism by denaturing gradient gel electrophoresis. Hum Mol Genet 1992;1:341-4.
6. Akane A, Yoshimura S, Yoshida M, Okii Y, Watabiki T, Matsubara K, et al. ABO genotyping following a single PCR amplification. J Forensic Sci 1996;41:272-4.
7. Gassner C, Schmarda A, Nussbaumer W, Schonitzer D. ABO glycosyltransferase genotyping by polymerase chain reaction using sequence-specific primers. Blood 1996;88:1852-6.
8. Cho D, Jeon MJ, Oh BJ, Song JW, Shin MG, Shin JH, et al. A simplified ABO genotyping by allele-specific polymerase chain reaction. Korean J Lab Med 2005;25:123-8. (조덕, 전미정, 오봉준, 송정원, 신명근, 신종희 등. 대립유전자특이중합효소연쇄반응법에 의한 간편한 ABO 유전형 검사. 대한진단검사의학회지 2005;25:123-8.)
9. Li CX, Pan Q, Guo YG, Li Y, Gao HF, Zhang D, et al. Construction of a multiplex allele-specific PCR-based universal array (ASPUA) and its application to hearing loss screening. Hum Mutat 2008;29:306-14.

10. Sanchaisuriya K, Chunpanich S, Fucharoen G, Fucharoen S. Multiplex allele-specific PCR assay for differential diagnosis of Hb S, Hb D-Punjab and Hb Tak. Clin Chim Acta 2004;343:129-34.
11. Angelini A, Di Febbo C, Baccante G, Di Nisio M, Di Ilio C, Cuccurullo F, et al. Identification of three genetic risk factors for venous thrombosis using a multiplex allele-specific PCR assay: comparison of conventional and new alternative methods for the preparation of DNA from clinical samples. J Thromb Thrombolysis 2003;16:189-93.
12. Yip SP. Single-tube multiplex PCR-SSCP analysis distinguishes 7 common ABO alleles and readily identifies new alleles. Blood 2000; 95:1487-92.
13. Muddiman DC, Null AP, Hannis JC. Precise mass measurement of a double-stranded 500 base-pair (309 kDa) polymerase chain reaction product by negative ion electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Commun Mass Spectrom 1999;13:1201-4.
14. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Res 2001;29:E88-8.
15. Song SH, Chang HE, Ryu KC, Lee HJ, Park KU, Song J, et al. Analysis for eight ABO alleles in Korean population. Korean J Lab Med 2006;26:374-9. (송상훈, 장호은, 유광철, 이현정, 박경운, 송정한 등. 한국인에서 8가지 ABO 대립유전자의 빈도 분석. 대한진단검사의학회지 2006;26:374-9.)
16. Cho D, Kim SH, Jeon MJ, Choi KL, Kee SJ, Shin MG, et al. The serological and genetic basis of the cis-AB blood group in Korea. Vox Sang 2004;87:41-3.
17. Yang YG, Kim JY, Song YH, Kim DS. A novel buffer system, AnyDirect, can improve polymerase chain reaction from whole blood without DNA isolation. Clin Chim Acta 2007;380:112-7.
18. Yang YG, Kim JY, Soh MS, Kim DS. A simple and rapid gene amplification from Arabidopsis leaves using AnyDirect system. J Biochem Mol Biol 2007;40:444-7.
19. Yang YG, Song MK, Park SJ, Kim SW. Direct detection of Shigella flexneri and Salmonella typhimurium in human feces by real-time PCR. J Microbiol Biotechnol 2007;17:1616-21.
20. Chou Q, Russell M, Birch DE, Raymond J, Bloch W. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Res 1992;11:1717-23.
21. Mercier B, Gaucher C, Feugeas O, Mazurier C. Direct PCR from whole blood, without DNA extraction. Nucleic Acids Res 1990;18:5908.
22. Ohhara M, Kurosu Y, Esumi M. Direct PCR of whole blood and hair shafts by microwave treatment. Biotechniques 1994;17:726-8.
23. Panaccio M, Georgesz M, Lew AM. FoLT PCR: a simple PCR protocol for amplifying DNA directly from whole blood. Biotechniques 1993;14:238-43.
24. Nishimura N, Nakayama T, Tonoike H, Kojima K, Kato S. Direct polymerase chain reaction from whole blood without DNA isolation. Ann Clin Biochem 2000;37:674-80.