RESEARCH ARTICLE

Rapid ABO genotyping by high-speed droplet allele-specific PCR using crude samples

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Background: ABO genotyping has common tools for personal identification of forensic and transplantation field. We developed a new method based on a droplet allele-specific PCR (droplet-AS-PCR) that enabled rapid PCR amplification. We attempted rapid ABO genotyping using crude DNA isolated from dried blood and buccal cells.

Methods: We designed allele-specific primers for three SNPs (at nucleotides 261, 526, and 803) in exons 6 and 7 of the ABO gene. We pretreated dried blood and buccal cells with proteinase K, and obtained crude DNAs without DNA purification.

Results: Droplet-AS-PCR allowed specific amplification of the SNPs at the three loci using crude DNA, with results similar to those for DNA extracted from fresh peripheral blood. The sensitivity of the methods was 5%-10%. The genotyping of extracted DNA and crude DNA were completed within 8 and 9 minutes, respectively. The genotypes determined by the droplet-AS-PCR method were always consistent with those obtained by direct sequencing.

Conclusion: The droplet-AS-PCR method enabled rapid and specific amplification of three SNPs of the ABO gene from crude DNA treated with proteinase K. ABO genotyping by the droplet-AS-PCR has the potential to be applied to various fields including a forensic medicine and transplantation medical care.

KEYWORDS

ABO genotype, buccal cells, dried blood spot samples, droplet allele specific PCR, single nucleotide polymorphism

1 | INTRODUCTION

The ABO gene is located on chromosome 9 and is composed of seven exons that encode the glycosyltransferase that determines the ABO blood group type. The major alleles of the ABO gene are A, B, and O, and have various single nucleotide polymorphism (SNP) sites.¹ ABO genotyping has become a common tool for forensic investigation because the method does not require a fresh blood sample, in contrast to serological blood typing.

The ABO genotypes are determined by SNP profiles. The SNP profiles are made from the pattern of nucleotides at each SNP locus. Specific detection of the nucleotide at each SNP locus is needed to determine the pattern of SNPs. Several methods able to discriminate between alleles with a single nucleotide alteration have been proposed, based

on PCR-restriction fragment length polymorphism,² PCR-single strand confirmation polymorphism,³ and allele-specific PCR (AS-PCR).⁴ Among these methods, AS-PCR is superior in terms of specificity/sensitivity and protocols. However, conventional PCR methods require a long reaction time in which almost 1 hour is needed for PCR amplification alone.⁴⁻⁶

We previously developed a droplet-PCR machine, which has better thermal conductivity than the conventional PCR machine and enables rapid PCR amplification.⁷⁻¹⁰ Our droplet-PCR is applicable to various genetic tests.⁷⁻⁹ In addition, droplet-PCR methods using allele-specific primers (droplet-AS-PCR) can detect SNPs.^{10,11}

In the present study, we applied the droplet-AS-PCR assay to ABO genotyping, and examined its specificity/sensitivity using crude DNA prepared without a purification extraction step from buccal cells and dried blood spot samples.

2 | MATERIALS AND METHODS

2.1 | DNA extraction from peripheral blood (PB) samples

Genomic DNA was extracted from PB obtained from 29 healthy volunteers using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. This study was approved by the institutional review board of Shinshu University (No. 351).

2.2 | Real-time droplet-PCR machine

The novel droplet-PCR machine (SEIKO EPSON, Suwa, Nagano, Japan) has two heating blocks and a fluorescent detector FLE1000 (Nippon Sheet Glass Co., LTD., Tokyo, Japan; Figure 1). The two heating blocks consistently regulate the temperature of each end of the reaction tube during denaturation (high temperature) and annealing/extension (low temperature). To perform a rapid temperature transition, the reaction tube is filled with silicone oil, allowing the droplet of PCR mixture to move easily in the tube during the mechanical rotation of the machine. The droplet transfers from one end of the reaction tube to another owing to gravitation every thermal cycle. Therefore, one microliter of the PCR mixture in the droplet state is able to perform shuttle PCR in the reaction tube.

2.3 | Direct sequencing

The ABO genotypes at three SNP loci were confirmed using genomic DNA obtained from PB by direct sequencing in both directions on an automated DNA sequencer (3500 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA). The genomic DNA samples obtained from PB were used for evaluating

the specificity and sensitivity of the droplet-AS-PCR assay, using SNP genotype-specific primers and probes.

2.4 | Sample preparation for evaluation of sensitivity

The detection limit of the droplet-AS-PCR for each of the three SNP loci was examined using mixtures with genomic DNA (50 ng/ μ L) having specific alleles (261G of AB, 261 Δ G of OO, 526G of AB, or 803C of AB) and ones possessing alternative alleles (261 Δ G of OO, 261G of AB, 526C of OO, or 803G of OO), respectively. For 526C and 803G, the detection limit of the droplet-AS-PCR was examined using plasmid DNA (10^5 copy/ μ L). Ratios of the specific alleles in the mixtures were adjusted to 50%, 10%, 5%, 1%, 0.5%, and 0.1%.

2.5 | Finders Technology Associates (FTA) paper processing

A few drops of PB from healthy volunteers were applied to an FTA classic card (GE Healthcare UK Limited, Buckinghamshire, UK). After the blood spots were dried for at least 1 hour at room temperature, disks 2 mm in diameter were punched out from the dried material on the FTA cards using a sterile hole puncher (Harris Micro-Punch; Shunderson Communications Inc., Ottawa, ON, Canada). One or two disks were used for analysis.

2.6 | DNA preparation from buccal cells and dried blood spot samples

Buccal cells and dried blood spot samples were obtained from 10 healthy volunteers whose genotypes were predetermined by the direct sequencing method. Two volunteers had the AO genotype, four were OO, two were BO, and two were AB. Buccal cells and dried

FIGURE 1 A diagram of the droplet-PCR machine. The diagram of the droplet-PCR machine was modified from our previous reports.^{7,9,10}

blood spot samples were hemolyzed using the hemolytic reagent and treated with 20 mg/mL proteinase K (Roche, South San Francisco, CA, USA) in digestion buffer (50 mmol/L Tris-HCl pH 8.5, 100 mmol/L NaCl, 1 mmol/L EDTA, 0.5% (v/v) Tween-20, 0.5% (v/v) NP40, 20 mmol/L DTT) at 50°C for 1 minute, and then denatured at 95°C for 1 minute to inactivate the proteinase K.

2.7 | Design of primers and probes

We selected three SNPs (at nucleotides 261, 526, and 803) from exons 6 and 7 of the ABO gene to identify the A, B, and O alleles, according to a previous report.¹² For each allele, allele-specific primers (Table 1) were designed as previously.¹³⁻¹⁵ The allele-specific primers included an SNP-matched nucleotide at the extreme 3'-end and a template-mismatched nucleotide located between the -3 to -1 positions from the 3'-end. TaqMan probes were labeled with fluorescein amidite at the 5'-end nucleotide and quencher (Minor Groove Binder) at the 3'-end nucleotide.

2.8 | Construction of plasmids carrying 526C, 526G, 803G, or 803C

To construct plasmids carrying 526C/803G and 526G/803C, PCR products were obtained by amplification of DNA from an AB type healthy volunteer using forward primer (5'-GGCAGCTGTCTGCTGGAGGT-3') and reverse primer (5'-GTGGCAGGCCCTGGTGAGCCGCTGCAC-3'). The amplicon were cloned into pCR2.1-TOPO vectors using the TOPO TA Cloning Kit (Thermo Fisher Scientific). The nucleotide sequences of 526C/803G and 526G/803C plasmids were confirmed by direct sequencing from 5' and 3' directions on an automatic DNA sequencer (3500 Genetic Analyzer; Thermo Fisher Scientific).

2.9 | Droplet-AS-PCR

ABO genotyping was performed using a high-speed droplet-PCR machine.⁷⁻¹⁰ The droplet-AS-PCR reaction mixture contained

genomic DNA (50 ng), Platinum Taq DNA polymerase (Thermo Fisher Scientific), 800 nmol/L of each primer pair designed as above, 300 nmol/L TaqMan probe, and reaction buffer composed of Tris-HCl pH 9.0, KCl, and MgCl₂, in a total volume of 10 µL. One microliter of the reaction mixture was used for the droplet-AS-PCR assay. The reaction conditions used were: 95°C for 10 seconds; and 35-40 cycles of 95°C for 4 seconds and 60°C for 8 seconds for all loci except for 261G when using dried blood and buccal cells. The annealing temperature for 261G when using dried blood and buccal cells was 62°C. The droplet-AS-PCR assays for the three SNP loci were performed in duplicate measurements using two aliquots prepared from a single PB preparation. All the SNP types of the 29 volunteers were pre-determined by direct sequencing. We determined an arbitrary standard of 5.9 for judging positive amplification results when the fluorescence level obtained from the positive samples were over 3SD above the mean of those from negative (alternative SNP type) sample.¹⁰ When the fluorescence level of the amplification by droplet-AS-PCR was over 5.9 at two consecutive assay points, we judged it as positive.

2.10 | Criteria for assigning the ABO alleles based on the three SNPs

Interpretative criteria for the ABO alleles and genotypes were according to Lee et al.¹² The interpretation based on the three SNPs is listed in Table 2.

3 | RESULTS

3.1 | Specificity of the droplet-AS-PCR for ABO genotyping

Representative amplification plots are shown in Figure 2. Samples from AA and BB types were not available. In SNPs at position 261, the droplet-AS-PCR provided amplification for 261G in AO, BO, and AB types, but not in the OO type, where only 261ΔG was present.

TABLE 1 Sequences of primers and probes for droplet-AS-PCR

Region	Nucleotide position	Variation	Primers (5'-3')	Probes (5'-3')
Exon6	261	G	F: TAGGAAGGATGTCCTCGTGTG	FAM-AACATCGACATCCTCA-MGB
		ΔG	F: TAGGAAGGATGTCCTCGTGCTAC	
			R: GGTGGTGTCTGGAGCCTGA	
Exon7	526	C	F: AGCTGTCAGTGCTGGAGG TTC	FAM-ACGTGTCCATGCGC-MGB
		G	F: AGCTGTCAGTGCTGGAGG CGG	
			R: CGCTCGCAGAAGTCACTGATC	
	803	G	R: CCGACCCCCGAAGAGCC	FAM-ACGAGGGCGATTCTACTAC-MGB
		C	R: CCGACCCCCGAAG ACCG	
			F: GCCCCAGTCCCAGGCCTA	

FAM, fluorescein amidite; MGB, minor groove binder.

ΔG, a single nucleotide deletion G at nt 261. Polymorphic nucleotides are given in bold and mismatched nucleotides introduced are bold underlined. SNP nucleotide positions were selected according to the previous report.¹²

TABLE 2 Interpretative criteria for the ABO alleles and genotypes identified by droplet-AS-PCR

	261G	261ΔG	526C	526G	803G	803C
<i>Allele</i>						
A	+	–	+	–	+	–
B	+	–	–	+	–	+
O	–	+	+	–	+	–
<i>Genotype</i>						
AO	+	+	+	–	+	–
AA	+	–	+	–	+	–
BO	+	+	+	+	+	+
BB	+	–	–	+	–	+
OO	–	+	+	–	+	–
AB	+	–	+	+	+	+

+, positive amplification; –, no amplification.

The amplification for 261ΔG was observed in AO, BO, and OO types, but not in the AB type, where only 261G was present. For 526C and 803G, amplifications were detected in AO, BO, OO, and

AB genotypes, as expected. For the 526G and 803C, SNPs found in allele B, on the other hand, amplifications were detected in BO and AB types, but not in the AO or OO types. These amplification profiles were shown to be specific according to the criteria based on SNPs listed in Table 2. Using the AS-droplet PCR, 29 samples were genotyped as follows: 11 were AO; 3 BO, 12 OO; and 3 AB. All of the genotypes determined by the droplet-AS-PCR were concordant with the genotypes from the direct sequencing method. We found that all amplifications were positive, and genotypes could be determined within 8 minutes.

3.2 | Detection limit of droplet-AS-PCR for three SNP loci

By the serial dilution experiment, the droplet-AS-PCR could specifically detect 261G, 261ΔG, 526G, and 803C at allele ratios of 5%, 5%, 10%, and 5%, respectively. In this experiment, the AA and BB types were not available, so we evaluated the detection limit for 526C and 803G using plasmid DNA (10^5 copy/ μ L) instead of PB. The detection limit for 526C and 803G using plasmid DNA were 5% and 1%, respectively.

FIGURE 2 Specificity of the droplet-AS-PCR method for ABO genotyping using genomic DNA extracted from fresh PB. In SNPs at position 261, the droplet-AS-PCR provided amplification for 261G (Δ) in AO, BO, and AB types, but not in the OO type. Amplification for 261ΔG (○) was observed in AO, BO, and OO, but not in the AB type. Amplification for 526C (□) and 803G (◇) was detected in all genotypes tested. Amplification for 526G (×) and 803C (*) was detected in BO and AB types. All amplifications were observed within 8 minutes. The x-axis indicates the cycles and time of PCR; the y-axis indicates the fluorescence level (AU: arbitrary unit)

3.3 | Droplet-AS-PCR using crude DNA from buccal cells and dried blood spot samples

DNA concentration (260/280 ratio) of buccal cells and dried blood spot cells were 27.8–121.7 ng/ μ L (0.69–0.85) and 36.0–40.8 ng/ μ L (0.71–0.76), respectively. A representative experiment with the AO genotype is shown in Figure 3. The droplet-AS-PCR could amplify 261G, 261 Δ G, 526C, and 803G, which was the pattern of the AO genotype. The specific amplifications for all three SNP loci using crude DNA from buccal cells and from dried blood spot samples were all equivalent to the results obtained using PB (Figure 3). The genotyping results obtained from 10 healthy volunteers by droplet-AS-PCR were all in accordance with results from the direct sequencing method. ABO genotypes using the crude DNA were determined within 9 minutes.

4 | DISCUSSIONS

In this study, it was possible to determine ABO genotypes from crude DNA within 9 minutes using the droplet-AS-PCR method. Crude DNA samples were obtained from buccal cells or dried blood spots that were treated with 20 mg/mL proteinase K in digestion buffer at 50°C for 1 minute. The crude DNA preparations enabled determination of ABO genotypes without undesired background PCR amplification.

The droplet-AS-PCR method could provide specific amplification for the SNPs at positions 261, 526, and 803. The major ABO alleles in the Japanese population are A101, A102, A201, B101, O01, and O02. Among the 13 SNPs residing in exons 6–7 of the ABO gene,^{1,16} the three SNPs at positions 261, 526, and 803 are enough to identify the A, B, and O alleles which occur frequently in the Japanese population.^{6,12} The droplet-AS-PCR method described here was specific for

FIGURE 3 Comparison of droplet-AS-PCR results using crude DNA from buccal cells and dried blood spots and purified DNA from PB. Crude DNA from buccal cells and dried blood spot samples showed the same amplification of SNPs 261G (Δ), 261 Δ G ($\circ$), 526C ($\square$), and 803G ($\diamond$) as DNA extracted from PB. The SNP pattern was compatible with the AO genotype. All amplifications were observed within 9 minutes. The x-axis indicates the cycles and time of PCR; the y-axis indicates the fluorescence level (AU: arbitrary unit)

single nucleotide alterations, and utilized primers with a mismatched nucleotide located between the -3 to -1 positions relative to the 3' terminus of the primer,¹³⁻¹⁵ a feature that was shown to provide high specificity for amplification from the desired templates. Droplet-AS-PCR yielded the same genotypes as the direct sequencing method, suggesting that droplet-AS-PCR can become a reliable method for ABO genotyping.

Crude DNA samples were available in addition to purified DNA from fresh blood for testing the ability of droplet-AS-PCR to determine ABO genotypes. ABO genotyping is a common tool for personal identification in the forensic field. However, fresh blood samples are not always obtained, and ABO genotyping often has to be determined from samples left at the crime scene such as dried blood, hair, small tissue specimens obtained by scratching.¹² Dried blood such as blood stain has been especially important for forensic analysis of specimens of human origin. In addition to ABO genotyping, dried blood is used to estimate the time the crime occurred according to the ratio of 18 S rRNA to β -actin mRNA.¹⁷ We showed that crude DNA samples that were obtained from buccal cells and from dried blood spot samples could be used to determine the ABO genotype via droplet-AS-PCR.

Crude DNA from dried blood was as useful as DNA extracted from fresh PB samples to determine ABO genotypes. Conventional PCR sometimes causes false DNA amplification because it cannot strictly control the temperature shift from the denaturing to the extension phase, and vice versa.⁹ On the other hand, the droplet-AS-PCR method can correctly change the temperature for PCR testing in a few seconds, and can amplify a target DNA without non-specific amplification in the presence of impurities. Therefore, crude DNA is a suitable sample for PCR testing by the droplet-AS-PCR method.

The times needed for determining ABO genotypes by droplet-AS-PCR from DNA extracted from fresh PB and from crude DNA were <8 and 9 minutes, respectively. ABO genotyping has been used in clinical applications, for example, Paternity Test,¹⁸ fetal ABO genotyping in maternal plasma in fetal-maternal ABO incompatibility,¹⁹ predisposition to pathogenesis of thrombosis event²⁰ and the risk test of hepatocellular carcinoma.²¹ Especially, in fetal-maternal ABO incompatibility, maternal IgG antibody cause destruction of the fetal red cells and then result in fetal hemolysis. Rapid fetal ABO genotyping contribute to early prevention, diagnosis and treatment of hemolytic disease of the fetus and newborn. ABO genotyping by the droplet-AS-PCR with rapidity and simplicity will be substantially useful to predict and prevent disease.

The droplet-AS-PCR methods using TaqMan probe can also provide quantitative data comparable with the conventional real-time PCR.⁷ ABO genotype can be used as donor/recipient discrimination markers, which is useful especially in HLA-matched but ABO-incompatible transplantations.^{22,23} Quantitative assessment of chimerism following HSCT is important for evaluation of engraftment/relapse. Genetic marker widely used in the chimerism analysis is short tandem repeat (STR), which discriminate donor/recipient according to the number of repeat of STR loci. However, chimerism assessment based on STR-PCR analysis is semi-quantitative and requires multiple processes including PCR, dilution of the PCR products and capillary electrophoresis using

sequencer. Thus, chimerism analysis based on ABO genotype using our droplet-AS-PCR method is superior to that based on STR using conventional PCR in terms of simple and rapid procedure and quantifiability. The droplet-AS-PCR could specifically detect all SNPs at the ratio of 5% or less except for 526G. The difference of melting temperature (T_m) between SNP-specific primer and the opposite primer may influence the decrease in sensitivity at 526G. For application of the present assay for chimerism analysis in clinical setting, it is needed to increase sensitivity using primers including T_m -modulator such as locked nucleic acid which are able to suppress the difference of T_m between two primers.

In conclusion, Rapid droplet-AS-PCR was able to determine ABO genotypes that are personal identification markers. The high speed, robustness, specificity, and sensitivity of the method are superior to those of other methods used for genetic testing in clinical laboratories. The positive features of droplet-AS-PCR should make the ABO genotyping widely used in the clinical setting.

REFERENCES

- Ogasawara K, Bannai M, Saitou N, et al. Extensive polymorphism of ABO blood group gene: three major lineages of the alleles for the common ABO phenotypes. *Hum Genet.* 1996;97:777-783.
- Ishida K, Zhu B, Sakoda S, et al. Significance of DNA analysis for determination of ABO blood groups from hair and nail of decomposed human remains: a comparison with phenotyping by the absorption-elution method. *Leg Med (Tokyo).* 2000;2:212-215.
- Downing J, Darke C. A modified PCR-SSP method for the identification of ABO blood group antigens. *Eur J Immunogenet.* 2003;30:295-298.
- Muro T, Fujihara J, Imamura S, et al. Determination of ABO genotypes by real-time PCR using allele-specific primers. *Leg Med (Tokyo).* 2012;14:47-50.
- Premasathan A, Ng J, Kanthaswamy S, et al. Molecular ABO phenotyping in cynomolgus macaques using real-time quantitative PCR. *Tissue Antigens.* 2012;80:363-367.
- Lee K, Park HC, An S, et al. A new method for ABO genotyping using fluorescence melting curve analysis based on peptide nucleic acid probes. *Leg Med (Tokyo).* 2015;17:334-339.
- Matsuda K, Yamaguchi A, Taira C, et al. A novel high-speed droplet-polymerase chain reaction can detect human influenza virus in less than 30 min. *Clin Chim Acta.* 2012;413:1742-1745.
- Sueki A, Matsuda K, Taira C, et al. Rapid detection of PML-RARA fusion gene by novel high-speed droplet-reverse transcriptase-polymerase chain reaction: possibility for molecular diagnosis without lagging behind the morphological analyses. *Clin Chim Acta.* 2013;415:276-278.
- Yamaguchi A, Matsuda K, Sueki A, et al. Development of a rapid and sensitive one-step reverse transcription-nested polymerase chain reaction in a single tube using the droplet-polymerase chain reaction machine. *Clin Chim Acta.* 2015;448:150-154.
- Taira C, Matsuda K, Yamaguchi A, et al. Novel high-speed droplet-allele specific-polymerase chain reaction: application in the rapid genotyping of single nucleotide polymorphisms. *Clin Chim Acta.* 2013;424:39-46.
- Taira C, Matsuda K, Yamaguchi A, et al. Rapid single nucleotide polymorphism based method for hematopoietic chimerism analysis and monitoring using high-speed droplet allele-specific PCR and allele-specific quantitative PCR. *Clin Chim Acta.* 2015;445:101-106.
- Lee HY, Park MJ, Kim NY, Yang WI, Shin KJ. Rapid direct PCR for ABO blood typing. *J Forensic Sci.* 2011;56:S179-S182.

13. Matsuda K, Sakashita K, Taira C, et al. Quantitative assessment of PTPN11 or RAS mutations at the neonatal period and during the clinical course in patients with juvenile myelomonocytic leukaemia. *Br J Haematol*. 2010;148:593-599.
14. Taira C, Matsuda K, Kamijyo Y, et al. Quantitative monitoring of single nucleotide mutations by allele-specific quantitative PCR can be used for the assessment of minimal residual disease in patients with hematological malignancies throughout their clinical course. *Clin Chim Acta*. 2011;412:53-58.
15. Taira C, Matsuda K, Saito S, et al. Application of allele-specific quantitative PCR using genomic DNA to monitor minimal residual disease based on mutant gene levels following allogeneic hematopoietic stem cell transplantation in patients with hematological malignancies: comparison of mutant levels with autologous DNA percentage by short tandem repeat-PCR. *Clin Chim Acta*. 2012;413:516-519.
16. Maeda K, Nakamura S, Murakami C, et al. ABO genotyping by TaqMan assay and allele frequencies in a Japanese population. *Leg Med (Tokyo)*. 2013;15:57-60.
17. Anderson S, Howard B, Hobbs GR, Bishop CP. A method for determining the age of a bloodstain. *Forensic Sci Int*. 2005;148:37-45.
18. Bugert P, Rink G, Kemp K, Klüter H. Blood group ABO genotyping in paternity testing. *Transfus Med Hemother*. 2012;39:182-186.
19. Song W, Zhou S, Shao L, Wang N, Pan L, Yu W. Non-invasive fetal ABO genotyping in maternal plasma using real-time PCR. *Clin Chem Lab Med*. 2015;53:1943-1950.
20. Sabino Ade P, Ribeiro DD, Dominghetti CP, et al. ABO blood group polymorphisms and risk for ischemic stroke and peripheral arterial disease. *Mol Biol Rep*. 2014;41:1771-1777.
21. Shim HJ, Lee R, Shin MH, et al. Association between ABO genotype and risk of hepatocellular carcinoma in Koreans. *Asian Pac J Cancer Prev*. 2015;16:2771-2775.
22. Flegel WA. ABO genotyping: the quest for clinical applications. *Blood Transfus*. 2013;11:6-9.
23. Liu F, Li G, Mao X, Hu L. ABO chimerism determined by real-time polymerase chain reaction analysis after ABO-incompatible haematopoietic stem cell transplantation. *Blood Transfus*. 2013;11:43-52.

How to cite this article: Taira C, Matsuda K, Takeichi N, et al. Rapid ABO genotyping by high-speed droplet allele-specific PCR using crude samples. *J Clin Lab Anal*. 2018;32:e22196. <https://doi.org/10.1002/jcla.22196>