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Research article

Dual approaches in pharmacogenetics: Developing PCR-SSP and RT-PCR methods for HLA-B*13:01 screening to prevent dapsone and Co-trimoxazole SCARs

Jirapat Attapong [a,c], Chollanot Kaset [b,c], Nontaya Nakkam [d], Wichittra Tassaneeyakul [d], Nuanjun Wichukchinda [e], Sirinart Chomean [b,c,*]

[a] Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand
[b] Department of Medical Technology, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand
[c] Thammasat University Research Unit in Medical Technology and Precision Medicine Innovation, Thailand
[d] Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[e] Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

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ABSTRACT

Dapsone and co-trimoxazole are potent antibiotics for treating various infections and inflammations. However, several studies reported the strongly association between severe cutaneous adverse drug reactions (SCARs) to both drugs and the HLA-B*13:01 allele. Rapid and reliable screening for the HLA-B*13:01 allele can mitigate the risk of dapsone-induced SCARs. We developed two methods, multiplex sequence-specific primer PCR (PCR-SSP) and real-time PCR (RT-PCR), tailored for different clinical settings. These methods were optimized to minimize false positives among the Thai population. Clinical validation demonstrated excellent reproducibility, with both methods showing 100 % concordance in repeated tests. PCR-SSP achieved a limit of detection as low as 100 pg of genomic DNA, while RT-PCR reached 1 pg. Overall statistical accuracy was 100.00 % (95 % CI: 98.18 %–100.00 %). Screening for drug-related HLA alleles is crucial for reducing mortality from severe cutaneous adverse drug reactions, especially dapsone hypersensitivity syndrome (DHS) and dapsone-induced hypersensitivity reactions (DIHRs). Our screening approach for dapsone can also be extended to co-trimoxazole, representing a significant advancement in personalized medicine and preemptive pharmacogenetic testing for tailored patient care and safety, albeit further validation in diverse ethnic populations is warranted to ensure universal applicability.

1. Introduction

Dapsone, chemically known as 4,4'-Sulfonyldianiline, is a significant drug in the sulfone category, widely used for treating leprosy, acne vulgaris, and *Pneumocystis carinii* pneumonia [1,2]. Its mechanism involves inhibiting dihydropteroate synthetase in the folic acid synthesis pathway and suppressing reactive oxygen species (ROS) formation. Recent research has extended its potential to neurodegenerative diseases, demonstrating antioxidant, anti-excitotoxic, and antiapoptotic activities [3]. However, dapsone is also associated with serious side effects, including fever, rashes, hematologic and hepatic disorders, and in severe cases, dapsone

* Corresponding author. Thammasat University Research Unit in Medical Technology and Precision Medicine Innovation, Thailand.
E-mail address: sirinat.c@allied.tu.ac.th (S. Chomean).

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hypersensitivity syndrome (DHS) or dapsone-induced hypersensitivity reactions (DIHRs), which have a mortality rate of 9.9 % [4]. Co-trimoxazole, a combination of sulfamethoxazole and trimethoprim, is effective against a range of bacterial infections. It acts by inhibiting bacterial folic acid synthesis. Despite its therapeutic benefits, it poses risks for hypersensitivity reactions, especially in HIV-infected patients, ranging from mild skin rashes to severe conditions like Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [5,6]. Emerging studies have demonstrated a significant correlation between the HLA-B*13:01 allele and severe cutaneous adverse reactions (SCARs) to drugs like dapsone and co-trimoxazole [6]. This finding is particularly relevant in diverse Asian populations and emphasizes the need for HLA-B*13:01 screening before prescribing these drugs [7–12].

The identification of predictive markers for DHS or DIHRs has led to focusing on the dapsone metabolism pathway, involving CYP2C9, CYP2C19, and CYP3A4 enzymes [13]. However, the most significant association has been found with the HLA-B*13:01 allele. Therefore, HLA-B*13:01 was identified as the predictive marker for dapsone-induced severe cutaneous adverse reactions (SCARs) in Thai patients [7]. Traditional HLA genotyping methods, like serological approaches and sequence-specific primer PCR (PCR-SSP), have limitations in resolution and throughput [14]. The advent of real-time PCR (RT-PCR) offers a rapid, robust, and cost-effective alternative, ideal for detecting HLA alleles in minimal genomic DNA concentrations [14–20]. This advancement is crucial in preventing

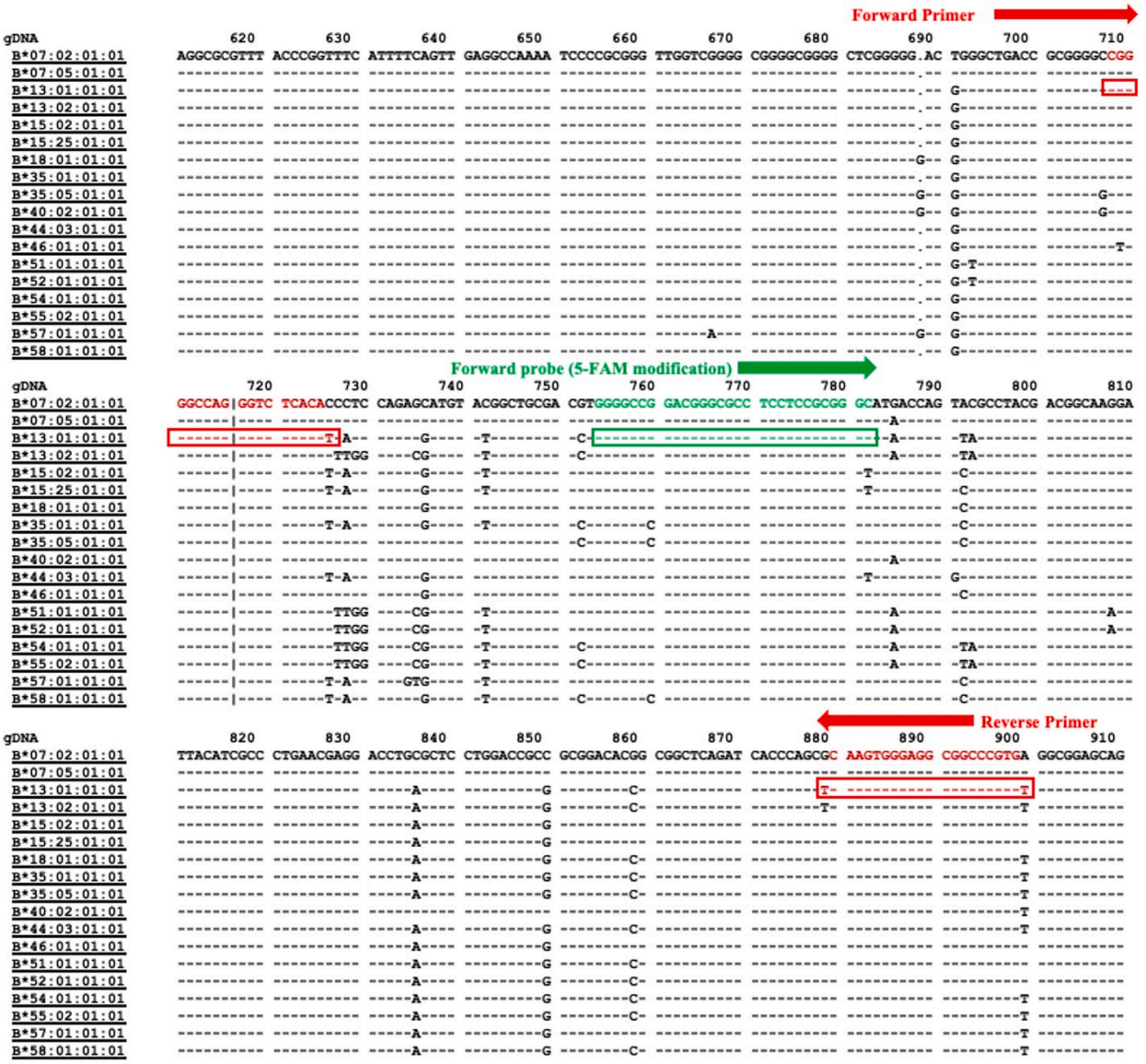

Fig. 1. Illustrates the binding sites of the primer and probe sets targeting specific regions, as referenced from the IPD-IMGT/HLA database. To mitigate the risk of false positives, alignments were made with common alleles (those having more than 1 % frequency) found in the Thai population. The primer set for the HLA-B*13:01 targets the exon 3 region, producing an amplicon of 193 base pairs. The alleles likely to be amplified are HLA-B*13:01. The probe for detecting this amplicon is positioned behind the forward primer and modified with 5'-carboxyfluorescein (5'-FAM).

SCARs and enhancing healthcare cost-effectiveness, as demonstrated in various studies focusing on HLA alleles associated to drug adverse effects [16–20].

Our study aims to develop two distinct methodologies for HLA-B*13:01 allele screening, catering to different clinical settings. The first method, a PCR-SSP technique, is designed for low-throughput laboratories with limited resources. The second, a real-time PCR method, is tailored for high-throughput scenarios in well-equipped hospitals. These approaches are crucial in preventing dapsone-induced SCARs and reducing mortality cases associated with these reactions. The adaptation of these methods for diverse clinical contexts marks a significant step in personalized medicine and the management of drug-induced health complications.

2. Materials and methods

EDTA (Ethylenediaminetetraacetic acid)- anticoagulated blood samples were collected from the blood bank unit at Thammasat university hospital for this study. Initially, a set of 30 samples (n = 30) was selected for the purpose of method optimization. This set included 8 HLA-B*13:01 positive samples, serving as positive controls. Subsequently, a larger group comprising 201 samples (n = 201) was utilized to evaluate the performance of the developed method. The allele frequency of HLA-B*13:01 in the Thai population is approximately 6.95 % [21], indicating that a minimum of 100 samples were necessary for an effective evaluation of the method's performance. This study utilized leftover EDTA blood samples from routine laboratory procedures, with all samples anonymized (blind HN) and devoid of any personal history. Given the nature of the samples and their use, the Proof of Ethics approval represents a certificate of exemption. This research received approval from the Human Research Ethics Committee of Thammasat University (Science), known as HREC-TUSc, with the Certificate of Ethical Approval number 005/2566-66AH026.

2.1. Genomic DNA extraction and purification

Genomic DNA was extracted from EDTA-treated peripheral blood samples using the Wizard® Genomic DNA Purification Kit (Promega, USA). Subsequently, the concentration of the extracted DNA was measured using a NanoDrop™ One Spectrophotometer (Thermo Fisher Scientific, USA). The concentration of the DNA samples exceeded 50 ng/μL. Additionally, the purity of the genomic DNA, assessed by the A260/A280 and A260/A230 ratios, was found to be over 1.8 and 1.5, respectively. For method optimization, the genomic DNA was diluted to a concentration of 20 ng/μL. In contrast, the genomic DNA extracted from the clinical samples was not diluted, to effectively evaluate the performance of the method.

2.2. Primer and probe design

Primers and probes were designed aligning with the IPD-IMGT/HLA database (https://www.ebi.ac.uk/ipd/imgt/hla/), using the HLA-B*07:02 sequence as a reference and comparing it against common HLA alleles found in the Thai population with an allele frequency of over 1 % [22], as illustrated in Fig. 1. The specificity and characteristics of these primers and probes, such as product size, melting temperature, and 3' self-complementarity, were verified using PRIMER-BLAST on the NCBI database (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The sequences and relevant parameters of the primers and probes are detailed in Table 1.

In this study, primers were specifically crafted for two distinct methods: sequence-specific primer PCR (PCR-SSP) and real-time PCR (RT-PCR). The primer targeting the HLA-B*13:01 sequence was positioned at the exon 3 region of the HLA-B sequence, resulting in a product size of 193 base pairs. The tissue inhibitor of metalloproteinase (TIMP-1) gene was used as internal control gene with a product size of 817 and 88 base pairs in PCR-SSP and real-time PCR (RT-PCR), respectively. In RT-PCR method, The amplicon of the HLA-B*13:01 gene was identified using a forward probe modified with 5'-carboxyfluorescein (5'-FAM), while the tissue inhibitor of metalloproteinase (TIMP-1) gene was detected using a forward probe modified with 5'-hexachlorofluorescein (5'-HEX). Both probes were modified with Black Hole Quencher 1 (BHQ1) at the 3' end.

Table 1
The sets of primer and probe specific for HLA-B*13:01 allele. The characteristics of these primers and probes were verified using PRIMER-BLAST on the NCBI database (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Name	Sequence (5' to 3')	Amplicon (bps)	T_m (C°)	%GC	Accession No.
HLA-B*13:01 specific primers and probe					
1301-RT-PCR (F)	CGGGGCCAGGGTCTCACAT	193	64.0	68.4	MN995311.1
1301-RT-PCR (R)	ACACGGGCCGCCTCCCACTTGA		70.5	68.2	
1301-Forward probe	**FAM**-GGCCGGACGGGCGCCTCCTCCGCGGGC-**BHQ1**		81.7	88.7	
Internal control primers and probe					
TIMP-1-SSP (F)	AGTTTCTCATTGCTGGTGAGGCACCGTCC	817	70.3	55.2	NG_012533.1
TIMP-1-SSP (R)	AGCCATCAGGGAACAGGCTTGGACTAG		67.8	55.5	
TIMP-1-RT (F)	CGCTCAGGCCCTGCCGCCAT	88	70.2	75.0	
TIMP-1-RT (R)	CGGTCCCTGCTGGGCCACCC		69.9	80.0	
TIMP-1 probe	**HEX**-CGCCGCAGATCCAGCGCCCAGAGAGACACC-**BHQ1**	–	76.3	70.0	–

2.3. HLA-B genotyping using sequence-specific oligonucleotide probes (PCR-SSO)

To assess the accuracy of our developed method, we employed sequence-specific oligonucleotide PCR (PCR-SSO) as the standard method. This procedure was conducted using the Luminex 100/200 Milliplex Analyzer system XYP SD, operated with Luminex xPONENT® software for LX100/LX200. For genotyping the HLA-B alleles in the samples, we used the LIFECODE® HLA-B eRES SSO Typing kit, lot number 3012346 (Immucor Inc., USA). The genotypes of HLA-B determined from the samples were subsequently analyzed using the MATCH IT DNA Software.

2.4. Performance evaluation of sequence-specific primer PCR (PCR-SSP) method

The optimal multiplex PCR was conducted in a total reaction volume of 20 μL, comprising 10 μL of PCR master mix (GoTaq® colorless master mix, Promega, USA), 500 nM of the HLA-B*13:01 primer, 250 nM of the TIMP-1 primer, sterile water, and genomic DNA. This PCR process was carried out in a Bio-Rad T100™ Thermal Cycler (Bio-Rad, USA) under the following optimal conditions: an initial start at 95 °C for 3 min, followed by 35 cycles of 95 °C for 15 s, 63 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The resulting amplicon was verified through agarose gel electrophoresis. The limit of detection was assessed using a series of ten-fold dilutions of genomic DNA, ranging from 100 ng to 10 fg. Furthermore, the method's specificity was evaluated using genomic DNAs carrying common alleles found in the Thai population, as depicted in Fig. 1. The concordance of the negative and positive results was then compared with the other two methods utilized in the study.

2.5. Performance evaluation of real-time PCR with TaqMan® probe method

The optimal multiplex real-time PCR was conducted using a total volume of 20 μL. This volume included 10 μL of PCR master mix (2X qPCRBIO Probe Mix Lo-ROX, PCR Biosystems, USA), 250 nM of the HLA-B*13:01 primer, 62.5 nM of the TIMP-1 primer, 250 nM of the HLA-B*13:01 probe, 250 nM of the TIMP-1 probe, sterile water, and genomic DNA. The PCR cycling process was carried out on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific, USA) using the QuantStudio 3/5 Real-Time PCR Software. The optimal cycling conditions were an initial start at 95 °C for 3 min, followed by 40 cycles at 95 °C for 5 s and 72 °C for 1 min 30 s. The QuantStudio Design Analysis software was used to set the amplification threshold and baseline adaptation algorithms. The limit of detection was determined through a series of ten-fold dilutions of genomic DNA, ranging from 100 ng to 10 fg. Additionally, the specificity of method was assessed using genomic DNAs that carry common alleles found in the Thai population. To differentiate between positive and negative results, the Ct cut-off value was established based on the carboxyfluorescein (5'-FAM) signal. This cut-off value represented the maximum Ct value that encompassed all HLA-B*13:01 positive samples while excluding signals from HLA-B*13:01 negative samples. The concordance of the negative and positive results obtained by this method was then compared with the results from the other two methods used in the study.

2.6. Statistical analysis

The performance parameters of the developed methods, including sensitivity, specificity, positive predictive value, and negative predictive value, were analyzed using MedCalc software version 22.013 (MedCalc Software Ltd, Ostend, Belgium) through its diagnostic test function. A 95 % confidence interval (CI) was used as the criterion for statistical significance.

3. Result

3.1. Optimization of the multiplex sequence-specific Primer PCR (PCR-SSP) method for HLA-B*13:01 screening

In our study, samples containing the HLA-B*13:01 allele were amplified using allele-specific primers for HLA-B*13:01, resulting in an amplicon size of 193 base pairs. Additionally, an amplicon of the tissue inhibitor of metalloproteinase (TIMP-1) gene, measuring 817 base pairs, served as an indicator of successful PCR reactions. Initially, the allele-specific primers for HLA-B*13:01 were capable of amplification at temperatures ranging from 60 to 72 °C, similar to the TIMP-1 primers. Subsequently, both primer pairs were combined in a single tube for the multiplex PCR reaction. The optimal annealing temperature was determined to be 72 °C. The final optimal concentrations were set at 500 nM for the HLA-B*13:01-specific allele and 250 nM for the TIMP-1 primers. The total duration for the PCR cycles was approximately 1 h and 16 min. Under these optimal conditions, the experimental results from 30 control samples (100 %) matched expectations. Specifically, 8 out of 30 samples carrying the HLA-B*13:01 allele produced amplicons of both 193 and 817 base pairs, while the remaining 22 samples, which did not carry the HLA-B*13:01 allele, yielded only the 193 base pairs amplicon.

3.2. Evaluation of the performance of the developed multiplex sequence-specific Primer PCR (PCR-SSP) method for HLA-B*13:01 screening

To assess the performance of our developed multiplex PCR-SSP method, we screened 201 samples and compared the results with those obtained using the PCR-SSO/Luminex method. Of these samples, 27 were found to carry the HLA-B*13:01 allele, while the remaining 174 did not. Notably, within the 174 samples, 2 were identified as carrying the HLA-B*13:02 allele, which has a sequence similar to that of the HLA-B*13:01 allele. Screening results using our multiplex PCR-SSP method showed that 27 samples tested positive (displaying both 193 and 817 base pairs) and 174 samples tested negative (exhibiting only the 817 base pair amplicon).

Diagnostic test parameters, taking into account the 6.95 % allele frequency of HLA-B*13:01 in the Thai population, were then analyzed. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the method were found to be 100.00 % (88.06 %–100.00 %), 100.00 % (97.88 %–100.00 %), 100.00 % (88.06 %–100.00 %), and 100.00 % (97.88 %–100.00 %), respectively, with an overall accuracy of 100.00 % (98.18 %–100.00 %). These results demonstrate the effectiveness of our method in HLA-B*13:01 screening.

To further investigate the limit of detection (LOD) of our multiplex PCR-SSP method, a series of ten-fold dilutions of samples carrying the HLA-B*13:01 allele were tested, ranging from 100 ng to 10 fg. As indicated in Fig. 2, our method required a minimum of 100 pg of genomic DNA for effective HLA-B*13:01 screening. In addition, to confirm the specificity of our method, we amplified 13 samples carrying common alleles in the Thai population, as shown in Fig. 3. The results matched expectation; the samples not carried HLA-B*13:01 allele yielded negative result. Notably, HLA-B*13:02, which has similar sequence with HLA-B*13:01 (726: T > C), was not amplified, highlighting the high specificity of our method.

3.3. Optimization of the multiplex real-time PCR (RT-PCR) method for detecting HLA-B*13:01

In this experiment, the tissue inhibitor of metalloproteinase (TIMP-1) gene was utilized as an internal control to confirm the success of the PCR reaction. Consequently, all samples were expected to produce a HEX signal, modified at the 5' end of the TIMP-1 probe. Samples containing the HLA-B*13:01 allele were amplified with an allele-specific primer, generating a FAM signal, similarly modified at the 5' end of the HLA-B*13:01 probe. Therefore, a positive result would show both HEX and FAM signals, while a negative result would only exhibit the HEX signal.

The optimization process began with identifying the optimal annealing temperature. The most effective temperature for both genes was determined to be 72 °C, as this temperature yielded a high probe signal without interference from the non-template control (NTC). This temperature was also found to be optimal for the multiplex real-time PCR. Distinct peak characteristics were designed for the two genes. The ΔRn of the HLA-B*13:01 was set to provide a clearer and higher signal than the internal control. The optimal final concentrations of the primers and probes were subsequently determined. For the HLA-B*13:01 gene, a primer concentration of 250 nM was optimal, providing the highest signal for the targeted gene (FAM) and maintaining a robust internal control (HEX) signal at a ΔRn threshold of 0.1. For the TIMP-1 gene, a primer concentration of 62.5 nM yielded a strong signal without affecting the targeted gene signal. Thus, the optimal primer concentrations were set at 250 nM for the HLA-B*13:01 gene and 62.5 nM for the TIMP-1 gene. The optimal probe concentration for both genes was established at 250 nM, balancing a high signal for the targeted HLA-B*13:01 gene with an adequate signal for the internal control TIMP-1 gene.

The total time for the PCR cycles was approximately 1 h and 6 min. Under these conditions, samples carrying the HLA-B*13:01 allele produced a FAM signal with an average Ct value of 25.60 ± 0.88 (Mean ± SD). The HEX signal, serving as the internal control, had an average Ct value of 24.76 ± 0.67 (Mean ± SD). Samples not carrying the HLA-B*13:01 allele showed only the HEX signal, with an average Ct value of 25.50 ± 0.87 (Mean ± SD), as illustrated in Supplementary Table S1. The ΔRn peaks for the HLA-B*13:01 gene ranged from 0.5 to 0.7, whereas those for the TIMP-1 gene ranged from 0.175 to 0.250. It was noted that the HLA-B*13:02 allele also yielded a negative result similar to samples not carried HLA-B*13:01, as illustrated in Fig. 4.

3.4. Evaluation of the performance of the developed real-time PCR (RT-PCR) method for detecting HLA-B*13:01

201 samples were amplified by our developed multiplex real-time PCR method. 27 samples provided both FAM and HEX signal as positive result while 174 samples provided only HEX signal as negative result. The average Ct value generated by FAM signal was 26.08

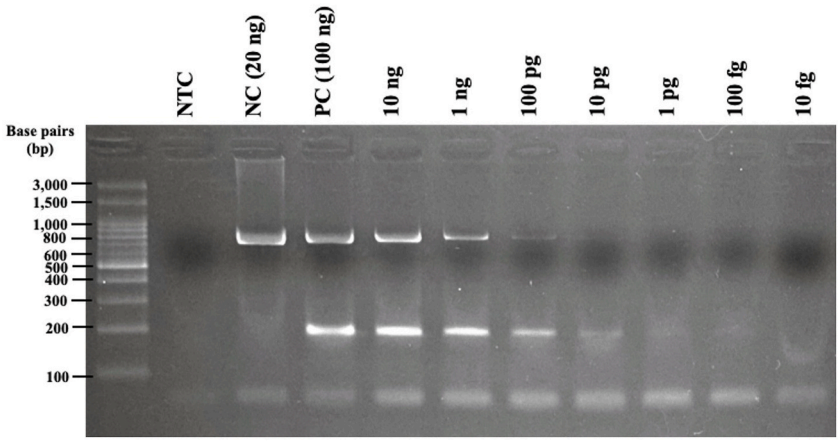

Fig. 2. The sensitivity evaluation of the PCR-SSP method, employing a series of ten-fold dilutions of genomic DNA. The labels in the figure denote NTC as the non-template control, PC as the positive control (HLA-B*13:01/58:01), and NC as the negative control (HLA-B*46:01/58:01). The genotype used for the ten-fold dilution series of genomic DNA was HLA-B*13:01/58:01.

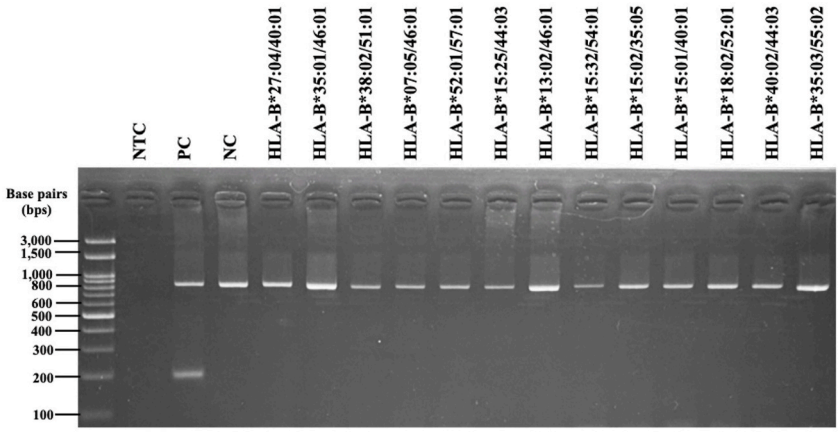

Fig. 3. The specificity evaluation of the PCR-SSP method using genomic DNA samples that carry common alleles found in the Thai population. In this figure, NTC represents the non-template control, PC indicates the positive control (HLA-B*13:01/58:01), and NC signifies the negative control (HLA-B*46:01/58:01).

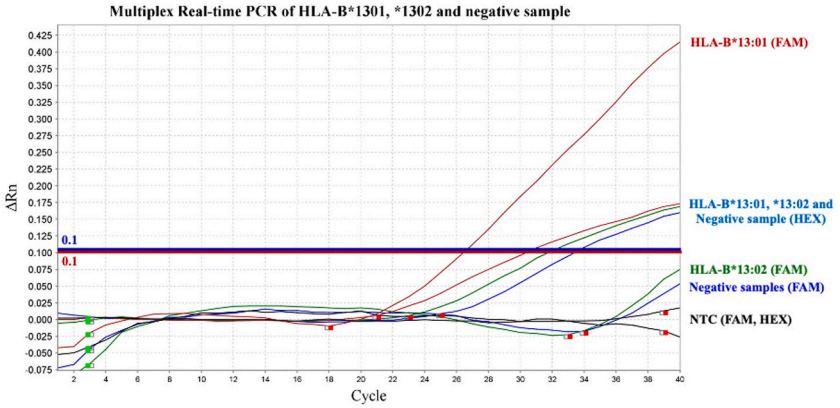

Fig. 4. the results obtained under optimal conditions. HLA-B*13:01 positive sample, represented by red line, generates both FAM and HEX signal, indicating amplification of both HLA-B*13:01 and internal control gene, while HLA-B*13:02 and HLA-B*13:01 negative samples, represented by green and blue line, generate only HEX signal, resulting from amplification of internal control gene. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

± 1.92 (Mean ± SD). Also, the average Ct value generated by HEX signal was 24.81 ± 1.63 (Mean ± SD), as illustrated in Supplementary Table S2-13. Then, the optimal Ct values were 30.00 as cut off for HLA-B*13:01 gene to define whether the samples provided positive or negative result. Also, the cut off TIMP-1 gene was determined at Ct value of 30.00 to ensure the success PCR reaction as well. Due to these cut off values, this method provided sensitivity, specificity, PPV and NPV of 100.00 % (88.06 %–100.000 %), 100.00 % (97.88 %–100.00 %), 100.00 % (88.06 %–100.00 %), and 100.00 % (97.88 %–100.00 %), respectively. The accuracy was 100.00 % (98.18 %–100.00 %). Therefore, our developed real-time PCR method has a good performance for HLA-B*13:01 detection.

Then, the series of ten-fold dilution genomic DNA were amplified ranged from 100 ng to 10 fg to investigate the limit of detection. As shown in Fig. 5, Ct values were affected by DNA concentration. For the HLA-B*13:01 gene, the Ct values of 100 ng, 10 ng, 1 ng, 100 pg and 10 pg were 26.06, 28.69, 29.23, 32.35 and 34.87 respectively (Fig. 5A). Then, the FAM signal dropped at DNA concentration of 1 pg with Ct values of 36.71. In the same way, the Ct values of TIMP-1 gene were 22.46, 25.37, 28.09, 31.40 and 34.46 to DNA concentration of 100 ng, 10 ng, 1 ng, 100 pg and 10 pg (Fig. 5B). Also, HEX signal dropped at DNA concentration of 1 pg with Ct values of 36.64 as well. Therefore, the limit of detection of our developed multiplex real-time PCR method was 1 pg or 0.001 ng. However, the very low genomic DNA concentration as 1 pg provided very low ΔRn which overlaid by NTC signal. To reduce the risk caused by misinterpretation, the least genomic DNA concentration was recommended at 10 pg or 0.01 ng with Ct value of both genes approximately 35.00 when ΔRn threshold was determined at 0.1.

Likewise, PCR-SSP performance evaluation, 13 Samples carried common alleles in Thai population were amplified to confirm specificity of this method. Fig. 6 A, B showed that sample not carried HLA-B*13:01 provided negative of FAM signal according to expectation. Also, HLA-B*13:02/46:01 provided negative result as well.

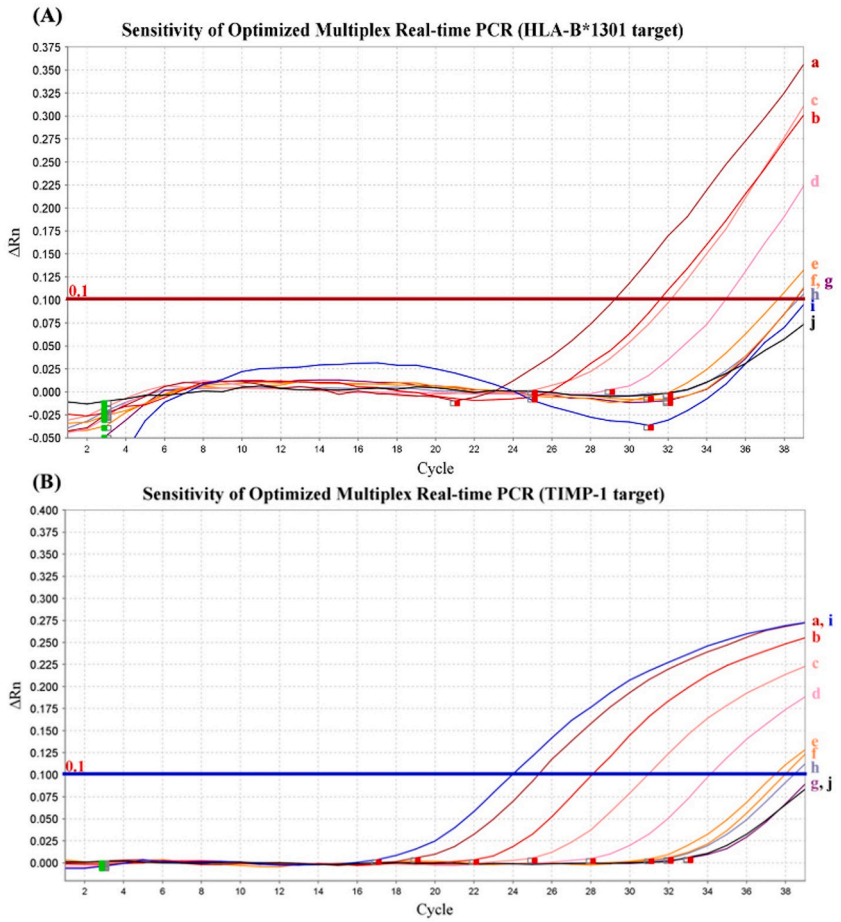

Fig. 5. The sensitivity evaluation of the real-time PCR method using a series of ten-fold dilutions of genomic DNA. In this figure, A) corresponds to the FAM signal peak, generated from the amplification of the HLA-B*13:01 gene, and B) represents the HEX signal, produced from the amplification of the TIMP-1 gene, serving as an internal control. The ten-fold dilution series of genomic DNA, specifically HLA-B*13:01/58:01, is represented as follows: a for 100 ng, b for 10 ng, c for 1 ng, d for 100 pg, e for 10 pg, f for 1 pg, g for 100 fg, h for 10 fg, i for the negative control (HLA-B*46:01/58:01), and j for the non-template control.

3.5. Testing the reproducibility of developed methods

We assessed the reproducibility of our developed methods by retesting 20 out of 201 (about 10 %) clinical samples. Both methods demonstrated 100 % concordance with previous results, thereby confirming their excellent reproducibility.

4. Discussion

Our study's core objective was to develop and validate methods for screening the HLA-B*13:01 allele, a pivotal factor in mitigating the risks of severe cutaneous adverse reactions (SCARs) induced by drugs such as dapsone and co-trimoxazole. By focusing on this allele, we aimed to reduce mortality rates, enhance the precision of treatments, and optimize healthcare resources. Our methodologies, multiplex PCR-SSP and real-time PCR, were specifically tailored for varying laboratory capacities. Our multiplex PCR-SSP and real-time PCR methods were tailored to suit different laboratory settings. The PCR-SSP was specifically designed for labs without access to costly equipment like real-time PCR machines, catering to those requiring low throughput. Conversely, our real-time PCR method was developed for larger hospitals equipped with such technology and handling a higher volume of samples, necessitating a high-throughput approach.

The primer sets in our study were designed to minimize false positives from common alleles found in the Thai population. In both multiplex PCR-SSP and real-time PCR methods, the HLA-B*13:01 specific primer was placed in the exon 3 region of the HLA-B gene. The forward primer was designed to provide a 3' mismatch with several alleles, including HLA-B*07:02, B*07:05, B*13:02, B*18:01, B*35:05, B*40:02, B*46:01, B*51:01, B*52:01, B*54:01, and B*55:02. Residual alleles not excluded by the forward primer, such as HLA-B*15:02, B*15:25, B*35:01, B*44:03, B*57:01, and B*58:01, were targeted by the reverse primer. Although the forward probe for the real-time PCR was not specific to HLA-B*13:01, the set of primers was specifically designed for HLA-B*13:01. Therefore, the

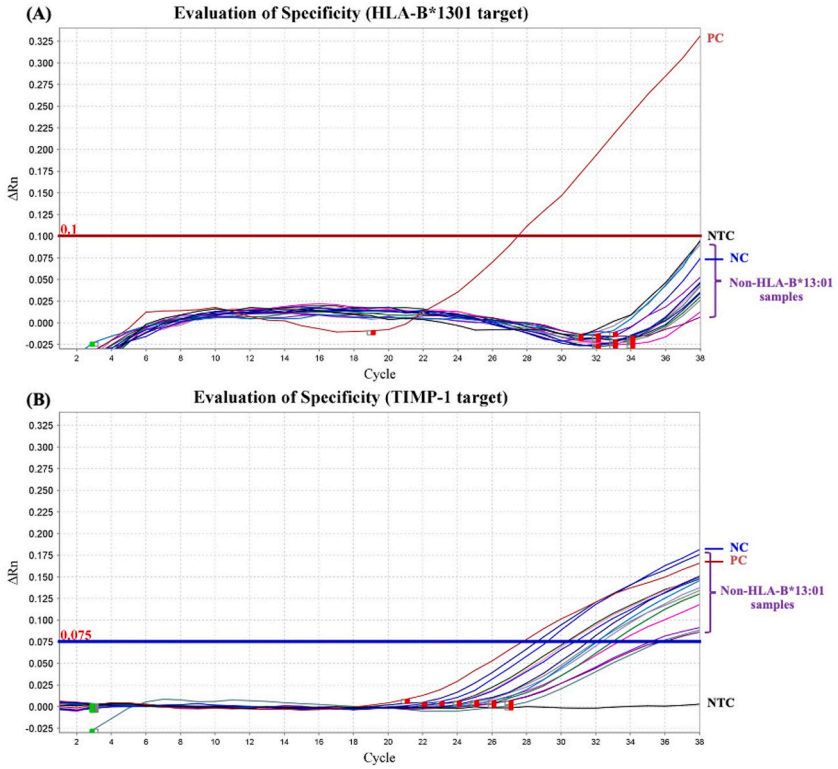

Fig. 6. The specificity evaluation of the real-time PCR method using genomic DNA containing common alleles found in the Thai population. In the figure, A) indicates the FAM signal peak, which is produced from the amplification of the HLA-B*13:01 gene, and B) shows the HEX signal, resulting from the amplification of TIMP-1, serving as the internal control gene. The designations are as follows: NTC for the non-template control, PC for the positive control (HLA-B*13:01/58:01), and NC for the negative control (HLA-B*46:01/58:01). Samples that did not carry HLA-B*13:01 alleles did not show amplification, as indicated by the absence of the FAM signal.

forward probe was used to detect the amplification of HLA-B*13:01 allele. Notably, the HLA-B*13:02 allele, sharing similarity with HLA-B*13:01, was not amplified, indicating the high specificity of these methods. Similar to the previous study, HLA-B*13:01 was distinguished from the common HLA-B alleles except for some rare alleles, such as HLA-B*13:06, HLA-B*13:12, HLA-B*13:22, and so on [20]. In the Thai population, HLA-B*13:03, HLA-B*13:10, and HLA-B*13:39 were found as rare alleles according to the previous report [21,22]. However, the relationship of those rare alleles with drug hypersensitivity reactions was not reported. Therefore, it may not be necessary to distinguish between those rare alleles in populations. Our methods showed strong performance, with a statistical accuracy of 100.00 % (95 % CI: 98.18 %–100.00 %), and low detection limits for both PCR-SSP and real-time PCR (Table 2).

Screening for HLA-B*13:01 is also critical for preventing SCARs induced by co-trimoxazole, commonly used in HIV/AIDS patients, in Thai, Malaysian, and Taiwanese populations [6,23]. Despite the additional screening cost of $11.84 USD per patient [24], it is economical compared to the costs associated with treating drug-induced SCARs [25], and it can guide the avoidance of hypersensitivity to co-trimoxazole and other drugs with the same predictive marker. Our methods were cost-effective, with screening tests priced at around $5 USD for PCR-SSP and $6.5 USD for real-time PCR.

Nonetheless, due to the varying prevalence of HLA-B*13:01 across ethnicities, re-validation of these methods is necessary for each population. Our screening strategy, based on the Thai population's HLA-B*13:01 prevalence, may not suit populations with different HLA alleles associated with dapsone-induced SCARs. Additionally, the potential amplification of rare alleles that share a similar sequence to HLA-B*13:01 raises concerns about false positives in ethnicities with a higher prevalence of those alleles. To address this, designing primers with specific 3' mismatches between these alleles and probes with sequences specific to HLA-B*13:01 would be beneficial for enhancing specificity.

5. Conclusion

In conclusion, our study successfully developed multiplex PCR-SSP and real-time PCR methods for the screening of the HLA-B*13:01 allele, crucial in preventing dapsone and co-trimoxazole-induced SCARs. These methods, characterized by high accuracy and low detection limits, are designed to minimize false positives, making them reliable and cost-effective for clinical use. While effective in the Thai population, their applicability to other ethnic groups with varying allele prevalence highlights the need for re-validation in diverse populations. Our research thus makes a significant contribution to personalized medicine, offering a practical

Table 2
Performance comparison of the developed methods analyzed using MedCalc software version 22.013 (MedCalc Software Ltd, Ostend, Belgium) through its diagnostic test function. Metrics include Area Under the Curve (AUC), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Limit of Detection (LOD).

	PCR-SSP performance (95 % CI)		Real-time PCR performance (95 % CI)	
Sensitivity	100.00 %	(88.06 %–100.00 %)	100.00 %	(88.06 %–100.00 %)
Specificity	100.00 %	(97.88 %–100.00 %)	100.00 %	(97.88 %–100.00 %)
AUC	1.00	(0.98–1.00)	1.00	(0.98–1.00)
PPV	100.00 %	(88.06 %–100.00 %)	100.00 %	(88.06 %–100.00 %)
NPV	100.00 %	(97.88 %–100.00 %)	100.00 %	(97.88 %–100.00 %)
Accuracy	100.00 %	(98.18 %–100.00 %)	100.00 %	(98.18 %–100.00 %)
LOD	100 pg		1 pg	

solution for enhancing the safety and efficacy of treatments involving dapsone and related drugs.

Data availability statement

Data have been deposited at Kaset, Chollanot (2024), "Raw data-Dual Approaches in Pharmacogenetics", Mendeley Data, V1, https://doi.org/10.17632/dx4xhd45p9.1.

CRediT authorship contribution statement

Jirapat Attapong: Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Chollanot Kaset:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Nontaya Nakkam:** Supervision, Resources. **Wichittra Tassaneeyakul:** Supervision, Resources, Conceptualization. **Nuanjun Wichukchinda:** Validation, Supervision, Investigation. **Sirinart Chomean:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34977.

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