New Testing Approach in HLA Genotyping Helps Overcome Barriers in Effective Clinical Practice

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BACKGROUND: Severe and potentially fatal hypersensitivity reactions to drugs, particularly antiepileptics, are clinically unpredictable. Recent evidence has revealed a strong and specific association between the implicated drug, the type of adverse reaction, and the particular HLA genotype. An urgent need exists for rapid diagnosis of HLA status to guide drug prescription; however, traditional HLA genotyping has a long turnaround time, is expensive, and is available only in specialized centers. We tested the feasibility of the loop-mediated isothermal amplification (LAMP)-based approach to detect a specific HLA genotype. As an example, we used B*1502, an HLA allele strongly associated with carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (C-SJS/TEN)3

CONCLUSIONS: Our study confirmed that the new LAMP method for detecting a specific HLA genotype is simple, inexpensive, accurate, and rapid, and may be of help in overcoming barriers in effective clinical practice.

Recent studies have demonstrated that certain HLA alleles are associated with greatly increased risks of severe adverse drug reactions (1, 2). In particular, HLA-B*1502 is strongly associated with carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (C-SJS/TEN)3

3 Nonstandard abbreviations: C-SJS/TEN, carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis; TAT, turnaround time; SBT, sequence-based typing; SSP-PCR, sequence-specific primer PCR; LAMP, loop-mediated isothermal amplification; LAMP-HB, LAMP-based method with heated blood samples.
Primer design. The LAMP assay primers were designed to target the region of exon 2 to exon 3 of the B*1502 allele of the HLA-B gene (major histocompatibility complex, class I, B; GenBank accession no. L42145). Two sets of primers (tubes A and B), each of which included a forward inner primer, a backward inner primer, 2 outer primers, and 1 or 2 loop primers, were used for the LAMP assay. Fig. 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol55/issue8 lists the primers used in this study and provides examples of other HLA-B alleles aligned with the B*1502 sequence to demonstrate the specificity of the primers. Most of the cross-reacting alleles are extremely rare, a feature that compromises the specificities only slightly (7).

Lamp reaction. The blood samples for LAMP reactions were first prepared by heat treatment. We diluted 10 μL of whole blood that had been collected into an EDTA-containing bottle with 90 μL water and heated it at 98 °C for 3 min. The LAMP reaction was carried out with the reaction mixture described by Notomi et al. (8, 9). The reaction mixture was incubated at 63 °C for 15–25 min for tube A and at 63 °C for 40–50 min for tube B. We detected the LAMP product by diluting SYBR Green I (Invitrogen) with 9 volumes of water and adding 1 μL to the reaction mixture. Positive and negative reactions were indicated by a green color and an orange color, respectively (Fig. 1). For quality control, a positive-control blood sample (B*1502 heterozygote) and a negative-control sample (B*1502-negative DNA) were performed with each batch of samples. A tested sample was considered positive for B*1502 when the 2 reaction tubes (both A and B) turned green and was considered negative for B*1502 when one or both of the tubes were orange.

PHASE I STUDY
The LAMP results for all 50 DNA samples (25 positive and 25 negative for B*1502) showed complete concordance with previous SBT results. The LAMP-HB results for the 200 frozen blood samples (30 positive and 170 negative for B*1502) were also completely concordant with previous SBT results. Of the 170 samples that tested negative for B*1502, 166 showed negative reactions in both the A and B tubes. A positive reaction (green color) was found in tube A for 1 sample.
and in tube B for the 3 other samples, suggesting the presence of expected cross-reactions and highlighting the desirability of using 2 sets of specific primers to enhance specificity. Hence, false-positive results due to cross-reactions were not observed in both tubes in the current study. Examination of the SBT data revealed that samples showing positive cross-reactions in tube A were actually from B*1515 carriers and that those showing such reactions in tube B were from carriers of B*1525.

PHASE II STUDY
In all 200 fresh blood samples, the B*1502 status indicated by LAMP-HB was identical to that concurrently obtained by SSP-PCR analysis. Both methods tested positive for B*1502 for 35 samples and negative for 165 samples. The mean (SD) for the TAT with the LAMP-HB method was 33.1 (10.8) min, and the 90th percentile for the TAT was 45 min.

In summary, our results with the new LAMP-based HLA-testing method with DNA from frozen or fresh blood samples were 100% concordant with those obtained by SBT or SSP-PCR, confirming that LAMP-HB is an accurate method for detecting a specific HLA genotype, B*1502. All positive and negative reactions were easily distinguished visually by a clear change of color.

DISCUSSION
Overwhelming evidence from multiple studies (3, 6, 10) strongly indicates that HLA-B*1502 is a necessary criterion (11) for the development of C-SJS/TEN. Despite a recommendation for screening for HLA-B*1502 in high-risk populations to avoid the use of carbamazepine in B*1502-positive patients (4, 12), its clinical implementation is prohibitively expensive and difficult because testing is costly and available only in specialized centers. In addition, the conventional test’s long TAT delays drug treatment and disease control. Although testing for HLA-B*1502 is most relevant for patients in Asia (where the B*1502 carrier rate is high (13)), many Asian countries have poor healthcare resources and cannot afford the conventional genotyping test (Table 1). Thus, an urgent need exists for a rapid diagnosis at a low cost.

To address this need, we developed and validated the LAMP assay, a method based on the principle of isothermal amplification of nucleic acids (8, 9), for the detection of specific genes. To ensure high specificities, we designed 2 sets of specific primers so that each set recognizes 6 specific regions on the target gene. With this test design, the LAMP approach is expected to be capable of detecting all specific HLA genotypes. To improve the visual interpretation of results, we added SYBR Green I, a DNA dye, to the reaction tubes at the end of experiments to clearly distinguish positive reactions (green color) from negative reactions (orange color) (Fig. 1). This use of a color change for detection was specifically tested with lipemic samples in another study (n = 10), which unequivocally indicated the correct reaction status. To enable the test to be performed easily outside the laboratory, to further reduce its cost, and to make test results available as soon as possible, we also investigated and confirmed the feasibility of direct use of heat-treated blood samples in the LAMP reaction mixtures without prior conventional DNA extraction. The TAT (from receiving the sample to reporting the result) was greatly shortened with the LAMP-HB method [a mean TAT of 33.1 (10.8) min and a TAT 90th percentile of 45 min in the phase II study, compared with the SSP-PCR TAT of 1–2 days]. Because of the high amplification efficiency, the test can even be applied to samples from individuals with severe leukopenia [mean white cell count, 0.56 (2.9) × 10⁹/L; n = 10]. In addition, hands-on times are greatly reduced, and no specialized training is required (Table 1). These advantages therefore make LAMP-HB ideally suited for development into a test kit for use in both clinical and bedside settings, particularly in underresourced communities. A cost–benefit analysis highlights the significant impacts on healthcare and social-resource allocation under different clinical scenarios (Table 1). With the existing test platforms, additional administrative resources (for communication of results, contacting/scheduling for a second visit, and so on) are required to arrange for the patient to obtain a prescription later, after the test result has become available. Thus, a delay in starting treatment is unavoidable with this approach. In contrast, because LAMP-HB produces a result quickly, medication can be started during the same patient visit. The cheaper test is expected to produce huge potential savings in costs, with faster results, shorter hospital stays, earlier disease control, and elimination of the diversion of resources for managing C-SJS/TEN (Table 1) (12, 14).

Although we did not encounter the situation in the present study, individuals carrying a theoretical combination of rare alleles that cross-react with B*1502 in both tube A and tube B (e.g., a carrier of both B*1515 and B*1525) will produce false-positive results for B*1502 (see Fig. 1 in the online Data Supplement). On the basis of reported population frequencies of these rare alleles, we estimate that the new LAMP-HB HLA test achieves a 100% sensitivity and a specificity of <1/100 but >99.9%. Thus, it is possible that if such extremely rare individuals develop disorders for which carbamazepine treatment is indicated, they might be denied the drug when tested with the LAMP approach.

In conclusion, our study of HLA-B*1502 may lay the foundation for a new approach to HLA genotyping for personalized medicine in pharmacogenetics.
immediate and direct benefits are safer drug use and more cost-effective clinical care. In addition, the development of a simple and inexpensive HLA-testing platform may reduce the costs of funding studies involving large sample sizes, thus facilitating the validation of findings involving rare alleles in HLA-association research. The success of this approach, as demonstrated in this study, may revolutionize HLA testing in both clinical and research arenas.

### Table 1. Cost–benefit analysis of introducing HLA testing and rapid diagnosis by LAMP-HB for the prevention of C-SJS/TEN.

<table>
<thead>
<tr>
<th></th>
<th>Noncompliance with FDA* guidelines</th>
<th>Compliance with FDA guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untested</td>
<td>Tested by SSP-PCR</td>
</tr>
<tr>
<td>Chance of developing C-SJS/TEN</td>
<td>1%–1.5%b</td>
<td>Not developed if B*1502 negative (NPV, 100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avoid CBZ if B*1502 positive</td>
</tr>
<tr>
<td>Mortality from C-SJS/TEN</td>
<td>Up to 30%</td>
<td></td>
</tr>
<tr>
<td>Morbidity from C-SJS/TEN</td>
<td>Pain, severe skin damage, residual scarring, eye complications, chronic xerostomia, and pulmonary complications</td>
<td></td>
</tr>
<tr>
<td>Costs of C-SJS/TEN management*</td>
<td>Hospital stay of 4–7 days in burn unit, immunoglobulin, antibiotics</td>
<td></td>
</tr>
<tr>
<td>Costs of C-SJS/TEN testing</td>
<td></td>
<td></td>
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<tr>
<td>Test costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagents</td>
<td>$51</td>
<td>$3.80</td>
</tr>
<tr>
<td>Labor</td>
<td>$77 (Trained staff × 4 h)</td>
<td>$9 (General staff × 1 h)</td>
</tr>
<tr>
<td>Total</td>
<td>$128</td>
<td>$12.80</td>
</tr>
<tr>
<td>Hands-on time</td>
<td>1 h</td>
<td>10 min</td>
</tr>
<tr>
<td>Test TAT</td>
<td>1–2 days; may be longer with referral from remote areas</td>
<td>1 h; test adaptable for use at bedside or in clinic</td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatients</td>
<td>Hospital stay of 1–2 days; delay in prescribing for and controlling the epilepsy</td>
<td>Early treatment and discharge</td>
</tr>
<tr>
<td>Outpatients</td>
<td>Required extra visits involve leaving work, loss of productivity, and traveling to and from clinic</td>
<td>Single visit</td>
</tr>
<tr>
<td></td>
<td>Time and costs for doctor consultation with patient</td>
<td>Early treatment</td>
</tr>
<tr>
<td></td>
<td>Additional resources and delay in prescribing for and controlling the epilepsy</td>
<td></td>
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</tbody>
</table>

* FDA, US Food and Drug Administration; NPV, negative predictive value; CBZ, carbamazepine.

b 0.20 (carrier rate =20% in Hong Kong) × positive predictive value [5% according to FDA and 7.7% in Taiwan study by Hung et al. (11)] = 1%–1.5%.

c Estimated total costs of hospital treatment for C-SJS/TEN, $4416; based on average national Medicare reimbursement in US in 2002 (Kagan et al. (14)).

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**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors’ Disclosures of Potential Conflicts of Interest:** No authors declared any potential conflicts of interest.

**Role of Sponsor:** The funding organizations played no role in the
design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.  

Acknowledgments: We thank David Wilmshurst for commenting on a draft of this report.

References


Previously published online at DOI: 10.1373/clinchem.2009.127894