External quality assessment of *HLA-B*5701* reporting: an international multicentre survey

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Objectives: *HLA-B*5701* strongly predicts abacavir hypersensitivity (HSR), but implementation of effective routine screening into clinical practice requires testing be practical and accurate. We tested the proficiency of *HLA-B*5701* typing among laboratories using sequence-specific primer PCR.

Design and methods: DNA panels (1 and 2) were distributed to seven laboratories (A to G) for blinded typing of the *HLA-B*5701* allele. Panel 1 (*n*=10 samples; *n*=7 laboratories) included 3 positives and other closely related *B17* subtypes (*B*5702*, *B*5703*, *B*5704* and *B*5801*). Panel 2 (*n*=96 samples; *n*=4 laboratories) included 36 positives among a broad spectrum of other B alleles. Two laboratories (A and B) also submitted 96 routine samples, typed by the same methodology, to the reference centre for additional analysis by sequence-based typing.

Results: All laboratories correctly typed panel 1 for *HLA-B*5701* carriage. Laboratories A, B and C identified *HLA-B*5701* alleles in panel 2 with 100% sensitivity and 100% specificity. Laboratory D reported one false negative, reportedly due to a sampling error. The results obtained for routine samples typed by laboratories A and B and those generated by the reference laboratory using sequencing were fully concordant.

Conclusions: Detection of *HLA-B*5701* alleles among laboratories was 100% specific and 99.4% sensitive, indicating that participating HIV testing laboratories were currently offering effective primary screening to identify individuals at high risk of abacavir HSR. Accurate reporting of *HLA-B*5701* status is critical for the safe administration of this drug and participation in quality assurance programmes by all sites who report *HLA-B*5701* status should be promoted.

Introduction

The long-term use of abacavir for the management of HIV has established its benefits as a safe [1] therapeutic option, with the major limitation being that 5–8% of white recipients develop a hypersensitivity reaction (HSR) within 6 weeks of commencing treatment [2–4]. The pathophysiology of abacavir-related HSR has been shown to be mediated via immunological pathways and to involve intracellular drug processing [3–5] that elicits a potent cellular response. Pharmacogenetic studies have revealed that abacavir HSR is highly associated with carriage of the *HLA-B*5701* allele. Thus, the risk of developing hypersensitivity can be individualized prospectively for each potential drug recipient, based on the carriage of this allele [6,7]. As rechallenge with abacavir after the development of true class I immunologically mediated HSR can result in a severe and even fatal reaction, screening to prevent the primary exposure of *HLA-B*5701*-positive individuals to abacavir has the potential to significantly increase the safety of this drug.

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The benefits of prospective genetic screening have already been demonstrated in the Western Australian HIV cohort, where *HLA-B*5701* genetic screening of 260 abacavir-naive patients demonstrated no cases of drug hypersensitivity among 148 *HLA-B*5701*-negative abacavir recipients [6]. Similar results have been found in a United Kingdom cohort study, where prospective *HLA-B*5701* screening among 561 abacavir-naive patients was associated with a significant reduction in the incidence of abacavir HSR to 0.5%, compared with an incidence of 6.2% among 300 patients who commenced abacavir before genetic screening was introduced [7]. In addition, a recently published French study of 137 consecutive patients who underwent *HLA-B*57/HLA-B*5701* screening using a two-step sequence-specific primer (SSP)-PCR assay found the incidence of clinically suspected abacavir HSR to be <1% after implementation of screening [8]. Observational studies have also supported that *HLA-B*5701* screening effects a decrease in the incidence of early discontinuation of abacavir in cases that do not fit criteria for clinical hypersensitivity. Clinical trials data, from two studies using patch testing to identify immunologically mediated abacavir HSR, the PREDICT-1 and SHAPE studies, should further inform the cost–benefit analyses of future pharmacogenetic screening strategies in racially diverse populations [9].

Given that accurate identification of *HLA-B*5701* is a prerequisite to the prevention of abacavir hypersensitivity, it is imperative that quality assurance programmes ensure accurate reporting of this allele. In 2006, the reference laboratory for the current study commenced national and international distribution of a SSP-PCR protocol and common prevalidated reagents (PCR buffer and primers) and controls to laboratories seeking to establish a method for the detection of the *HLA-B*5701* allele. Participating laboratories were mostly from tertiary-care, University-affiliated hospitals, and varied in terms of the regulatory frameworks within which they operated and their experience in the provision of genetic screening.

Details of the methodology recommended to the participating laboratories have been published elsewhere [10]. Briefly, the assay used SSP in a multiplex reaction to selectively amplify the *HLA-B*5701* allele, along with two differently sized PCR products indicating the presence of *B*57* alleles and a housekeeping or reference gene (human growth hormone, *HGH*) [10]. Amplicons were resolved by agarose electrophoresis and the banding patterns were used to assign *HLA-B*5701* status.

Proficiency in testing for *HLA-B*5701* status requires high sensitivity, because failure to detect the HLA-B*5701 allele could lead to the inappropriate prescription of abacavir to individuals at high risk of developing HSR. High specificity is also required to avoid inappropriately denying abacavir to individuals at low risk of HSR. Our quality control survey comprised three phases: phase one represented a pilot study involving the distribution of a small panel of samples for *HLA-B*5701* typing, phase two expanded the distribution to involve a more comprehensive panel of samples, and phase three assessed proficiency in detection of the *HLA-B*5701* allele for samples received by participants during clinical practice.

Materials and methods

Reagents and protocol

Participating laboratories from Canada (*n*=5), Germany (*n*=1) and Australia (*n*=1) were supplied with a common protocol for SSP-PCR detection of *HLA-B*5701*. Common, validated batches of PCR buffer, primer mix and *HLA-B*5701*-positive and *HLA-B*5701*-negative control DNA were also distributed to each laboratory (shipped at ambient temperature with an ice pack), as described below.

Phases one and two: assessment of *HLA-B*5701* detection using control panels of DNA

For the first two phases, participants were sent a panel of samples along with a request to report the carriage of *HLA-B*5701* alleles for each sample. For phase one, panel 1 (*n*=10 coded samples) was distributed to laboratories A–G and included three positives and other closely related *B17* subtypes (*B*5702*, *B*5703*, *B*5704* and *B*5801*). For this panel, the DNA was supplied in 10 µl volumes in individual tubes at a concentration of 40 ng/µl, together with a request to submit a digital image of the PCR products run on agarose gel and a description of any deviations from the recommended protocol. In phase two, panel 2 (*n*=95 coded samples of 20 ng/µl of DNA in 10 µl water and one sample of water) was distributed in sealed PCR plates to laboratories A–D and included 36 positives among a broad spectrum of other B alleles (Table 1). Result interpretation for both panels was based on the presence of three amplicons (growth hormone control and generic *HLA-B*57*- and *HLA-B*5701*-specific amplicons), as described previously [10].

Sequence-based typing (SBT) at the *HLA-B* locus for both panels of DNA was performed by the reference laboratory and used to assess the accuracy of results reported by participants.

Phase three: assessment of *HLA-B*5701* reporting for the first samples (*n*=96) received by participants through clinical practice

Phase three focused on assessing the accuracy of typing of samples that were received by participating

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laboratories as part of a prospective screening programme to identify individuals at high risk of abacavir HSR. The selection criterion for inclusion in the screening programme was based on the requirement of the individual to receive antiviral therapy for treatment of HIV infection. Current or previous antiretroviral treatment, immunological factors or demographic factors including race were not considered. To our knowledge the majority of clinicians reported screening all of their HIV-infected patients who were abacavir-naive, regardless of clinical or self-identified ethnicity. DNA from the first 96 patient samples that were received and prospectively typed for *HLA-B*5701* by SSP-PCR for laboratories A and B was aliquoted into 96-well PCR plates, sealed and shipped at ambient temperature to the reference laboratory in Australia.

The samples were de-identified and typed at the *HLA-B* locus by direct sequencing. Concordance of *HLA-B* locus typing results between SSP-PCR and sequencing data was assessed.

Results

Phase one

All laboratories (A–G) correctly reported carriage of *HLA-B*5701* alleles for panel 1, on the basis of concordance with SBT at the *HLA-B* locus. Images of the agarose gels that were used to report *HLA-B*5701* status, as well as details of the *HLA-B* alleles as determined by SBT, are shown for each sample of panel 1 in Figure 1.

Deviation from the protocol for laboratory A included omission of an initial spectrophotometric analysis of DNA and use of a mini gel for electrophoresis of PCR products (150 volts for 15 min). Deviations from the protocol for laboratory B included use of different reagents: 10× PCR buffer (Applied Biosystems, Foster City, CA, USA), 25 mM $MgCl_2$ (Applied Biosystems), 40 mM dNTPs mix (Introgene, Leiden, CA, the Netherlands) and Taq Gold (Applied Biosystems). Laboratory G used $MgCl_2$ at a final concentration of 1.5 mM, rather than 1.25 mM.

Phase two

Laboratories A, B and D reported the *HLA-B*5701* status for all samples of DNA in panel 2 (*n*=95). All participants correctly failed to generate a result for one sample that contained only water. Laboratory C had results for 92 of the 95 samples of DNA: two samples failed to amplify and one sample showed presence of the *HLA-B*5701* amplicon, but not the B*57 amplicon. The latter result was not classified as a false-positive result because failure to amplify the *HLA-B*57* amplicon breaches the criteria for assignment of a *B*5701* result; that is, the SSP-PCR protocol requires all *HLA-B*5701*-positive samples to also be positive for the generic *HLA-B*57* amplicon. This particular banding pattern result was the first to be reported, and we can provide no particular explanation for its occurrence.

Overall, carriage of the *HLA-B*5701* allele for panel 2, was reported with 100% sensitivity and 100% specificity by laboratories A, B and C. Laboratory D reported one false-negative *HLA-B*5701* result. This particular sample was initially amplified as a weak positive. During subsequent repeat testing a sampling error occurred: that is, the incorrect sample was retested. After the discordance in this result was identified, re-typing of the correct sample yielded the correct result.

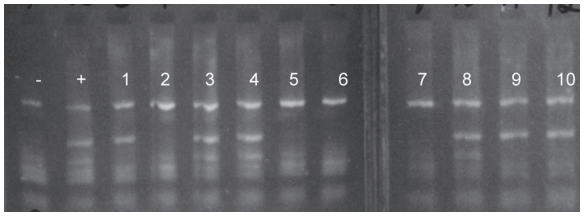
Table 1. Samples (*n*=96) in panel 2 carrying specific *HLA-B* alleles

HLA-B specificities	Samples, *n*
*B*5701*	36
*B*0702, 0744*	22
*B*080, B*0819N,B*0807*	9
*B*1301, B*1302*	4
*B*1402, B*1403,*	4
*B*1503*	6
*B*1801, B*1817, B*1817N*	3
*B*2705, B*2713*	1
*B*3501, B*3503, B*3533, B*3551, B*3505, B*3534, B*3542, B*3540N, B*3557*	5
*B*3701*	2
*B*3906*	2
*B*4001, B*4002*	4
*B*4101*	2
*B*4201*	3
*B*4402, B*4427, B*4415, B*4419N*	5
*B*4501, B*4507*	2
*B*4701*	3
*B*4901*	3
*B*5001*	1
*B*5101, B*5132, B*5130*	1
*B*5201, B*5207*	3
*B*5301*	1
*B*5501*	1
*B*5601*	1
*B*5703*	4
*B*5801, B*5811, B*5810, B*5810N, B*5802,*	16
*B*8101, B*8102*	1

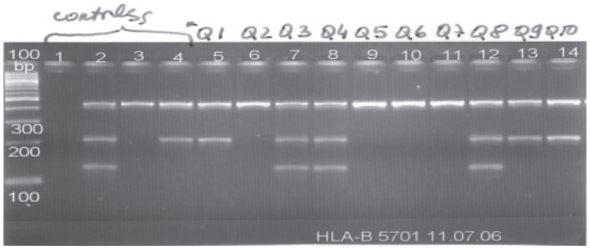
Phase three

Reporting of *HLA-B*5701* status, as performed for prospectively screening of routine samples (*n*=96) by laboratories A and B, was 100% concordant with the SBT of the *HLA-B* locus performed by the reference laboratory. The frequency of the *HLA-B*5701* allele, as typed by laboratory A and B, was 4 and 5%, respectively.

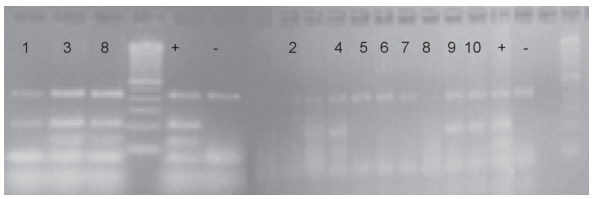
Figure 1. Agarose gel images of the electrophoresed sequence-specific primer PCR amplicons that were used by laboratories A–G to report *HLA-B*5701* status for panel 1



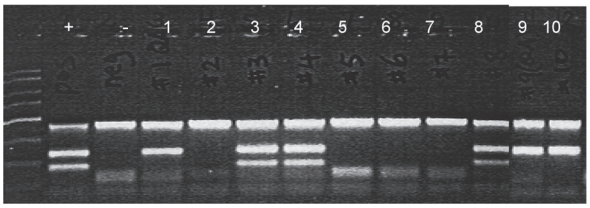
Laboratory A



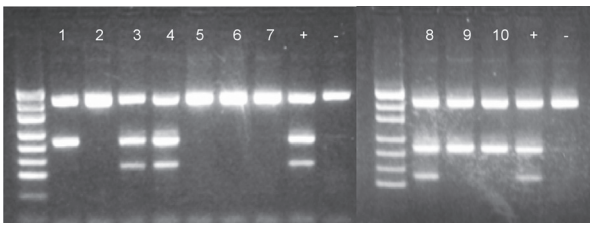
Laboratory B



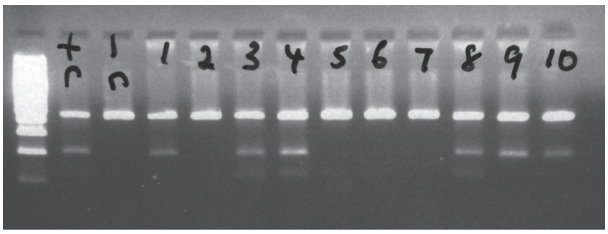
Laboratory C



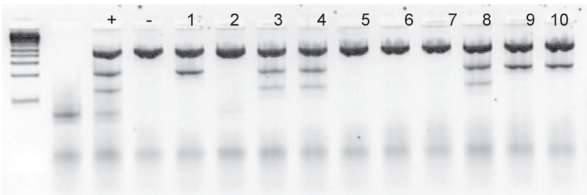
Laboratory D



Laboratory E



Laboratory F



Laboratory G

The samples are identified by numbers 1 to 10 for each gel image. Sequence-based typing of the human leukocyte antigen (HLA) B locus for these samples were as follows: (1) *B*5704/B*5301*, (2) *B*44/B*40*, (3) *B*5701,B*44*, (4) *B*5701/0801*, (5) *B*5801/B*4201*, (6) *B*5801/B*1502*, (7) *B*4403/B*40*, (8) *B*5701/B*3701*, (9) *B*5703/B*4001* and (10) *B*5702/B*1503*.

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Discussion

This study describes the implementation of a three-phase quality assurance survey to assess the proficiency of laboratories who had recently established a typing service for the *HLA-B*5701* allele. Seven laboratories completed the first phase, a pilot study comprising blinded typing of a 10-sample panel (panel 1) including three *HLA-B*5701* positive samples and seven other closely related *HLA-B* subtypes. For this aspect, *HLA-B*5701* typing results were fully concordant with sequence-based typing; that is, 100% specific and 100% sensitive. The results from this exercise, involving numerous laboratories of varying backgrounds, were promising and served as an impetus for more comprehensive assessment involving a larger panel.

Four of the seven laboratories participated in the second phase of the survey, involving the reporting of *HLA-B*5701* status for 96 samples including 36 *HLA-B*B5701* positive samples and a broad range of other *HLA-B* alleles. The data indicated a high degree of specificity (100%), but one of the four laboratories had a false-negative result. Nevertheless, the overall sensitivity, at 99.3% (143/144 *HLA-B*5701*-positive samples correctly identified), was high for panel 2 across the four sites. Although there is no room for complacency, these figures are promising and compare favourably to sensitivities of 95% and specificities of 64% achieved in other studies for typing of the *HLA-B27* allele in a clinical setting [11].

Two laboratories (A and B) participated in phase three of the study, which assessed the accuracy of reporting *HLA-B*5701* status for the first 96 samples that were requested for routine prospective screening by local medical practitioners in a clinical setting. For both participating laboratories, reporting was fully concordant with data generated independently by the reference laboratory, using SBT. The numbers of positive samples that were identified by laboratories A and B were 4 and 5%, respectively. Compared with phase two of the study, involving blinded typing of a DNA panel (2) enriched for *HLA-B*5701*, this aspect of the programme was not as highly powered to test for sensitivity to detect *HLA-B*5701*-positive samples. In phase three, the reported frequency of positive samples simply reflected the frequency of the allele in the local population. However, this phase was wider in scope, and tested additional variables related to the routine processing of samples and issuing of reports which might also affect the reported result, such as upstream sample receipt and handling, DNA extraction and evaluation, management of data integrity and temporal changes that might occur over the course of an extended reporting process. In this regard, the findings of phase three for laboratories A and B are encouraging and suggest a good level of proficiency for these participants in the establishment of a new service.

The SSP-PCR approach used by all participants in this study will also detect the closely related and rare HLA alleles *B*5708*, *B*5514*, *B*5710*, *B*5706* and *B*5814*. Because of the rarity of these alleles it has not been possible to clinically establish whether they are associated with class I immunologically mediated abacavir HSR. Furthermore, after sequencing the *HLA-B* locus for >1,000 samples (including the sequence data generated for phase three of the current study), the reference laboratory is yet to detect any such alleles. This suggests that the described SSP-PCR assay does not significantly contribute to false identification of *HLA-B*5701*-positive samples in the populations studied.

Although using a similar protocol, methodological details were individualised by each laboratory. Laboratories rarely share the same history of genetic typing or have identical approaches to the implementation of quality management systems. It is therefore implicit that the working environments of laboratories participating in this exercise also differed. That the results were consistent between laboratories suggests that the general approach, based on SSP-PCR, is relatively robust and is unaffected by these minor inter-laboratory differences.

It is important to emphasize that accurate reporting of the *HLA-B*5701* allele is a central safety issue for minimizing abacavir-related toxicity. This study represents the first of many steps in the validation of *HLA-B*5701* status reports issued by participating laboratories. The sample distribution exercises were simplistic in design, comprising panels of DNA prepared at only one laboratory. The findings of this study should therefore not be extrapolated as an assessment of all potential variations that could lead to poor results. Additional surveys are required to continue the process of accruing sufficient data to assign confidence intervals to the levels of sensitivity and specificity offered by the participating laboratories for typing of *HLA-B*5701*. Participants in the current exercise included some laboratories where HLA typing is not routinely done, and it is likely that other laboratories from diverse backgrounds might also establish typing services for *HLA-B*5701*. In this setting, future programmes which provide a more comprehensive picture of laboratory performance, by assessing other aspects of laboratory operations such as sample type and storage, DNA extraction methods and personnel training, would be ideal. The need for quality assurance programmes to monitor the global accuracy of *HLA-B*5701* status reports cannot be over-emphasized. This will be particularly applicable to the developing world

and resource-poor and racially diverse settings such as India and Northern Thailand, where the carriage rate of *HLA-B*5701* is high, as well as Africa, where there is a pressing need for less toxic drug regimens. Quality assurance programmes should serve to identify the best approaches, be they DNA- or antibody-based methods (for example, flow cytometry or simple agglutination cards), for specific laboratory settings. A continuing commitment to investing in quality assurance programmes, with increasing focus on clinically relevant biological material, should therefore be promoted in collaborative approaches to this expanding area of pharmacovigilance.

In summary, the results of this three-phase quality assurance programme for SSP-PCR-based screening for *HLA-B*5701* to prevent abacavir hypersensitivity reaction are reassuring and demonstrate that these laboratories can effectively provide the level of accuracy required for the successful implementation of prospective screening programmes. Furthermore, ongoing quality assurance programmes across diverse laboratories and methodologies will be useful and necessary to maintain these standards.

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