ABILITY OF A REAL TIME PCR TO DETECT FOR THE PRESENCE OF HLA B*57:01 ALLELE USING DNA EXTRACTED FROM WHOLE BLOOD DRIED SPOTS

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ABSTRACT

Background

Abacavir (ABC) is a NRTI recommended for the treatment of HIV-1 infection. It is a potent medication but has a limiting toxicity of hypersensitivity reaction (HSR), in 2–8% of treated patients. HSR to ABC is significantly associated with carriage of the HLA-B*57:01 allele and HLA-B*57:01 testing is recommended to be performed prior to ABC initiation.

Some real-time PCR assays were developed for HLA-B*57:01 testing but not have been validated using whole blood blotted. We assessed the ability of a real time PCR to detect for the presence of HLA B*57:01 allele using DNA extracted from whole blood spotted on blotters.

Materials and methods

A total of 200 genomic DNA samples were tested: 70 were HLA-B*57:01 allele positive and 130 HLA-B*57:01 allele negative. Whole blood belonged to HIV carriers, previously screened for HLA-B*57:01, was spotted on blotters and dry blood spots and stored at room temperature for 2 weeks before use.

Total DNA was extracted from 50 µl of whole blood spotted on blotters by using the EZ1 Advanced XL Qiagen instrument with the EZ1 DSP Virus Kit. DNA was measured spectrophotometrically and diluted to 10 ng/µl. Three primers target the HLA B*57:01 allele: HLA1 (5'-GTCTCACATCATCCAGGT-3'), HLA2 (5'-ATCCTTGCCGTCGTAGGCGG-3') and HLA3 (5'-ATCCTTGCCGTCGTAGGCAG-3') (Martin et al., 2005). The reverse primers HLA2 and HLA3 target the HLA B*57:0101 and B*57:0102 alleles, respectively, both coupled with the invariant forward primer HLA1, yielding a PCR product of 96 base pairs in length.

Results

Our results show that our assay used from DNA extracted from dried blood spots was 100% sensitive, detecting all HLA-B*57:01 allele-positive patients and 100 % specific, having no false-positive results achieving a 100% specificity and 100% sensitivity on this control panel.

Conclusion

Dried blood spots are an alternative specimen type for HIV drug resistance genotyping and HIV-1 diagnosis in patients in resource-limited settings but can also be used to detect the presence of HLA B*57:01 allele.

INTRODUCTION

- According to the latest WHO figures , HIV continues to be a major global public health issue, having claimed more than 35 million lives so far. In 2017, 940 000 people died from HIV-related causes globally. There were approximately 36.9 million people living with HIV at the end of 2017 with 1.8 million people becoming newly infected in 2017 globally. 59% of adults and 52% of children living with HIV were receiving lifelong antiretroviral therapy (ART) in 2017 and will require ongoing monitoring to ensure treatment continues to be efficacious.
- While CD4 cell count has traditionally been used to monitor patients on treatment in resource-limited settings, HIV viral load (VL) testing is increasingly recognized as the preferred tool for monitoring treatment efficacy, detecting treatment failure, and preventing misclassification of treatment responses and inappropriate switching of treatment regimens. However, despite recommendations by WHO in 2010 for countries to begin phasing in VL monitoring, current VL technology remains out of reach for the majority of patients in most resource-limited settings as standard HIV nucleic acid approaches depend on expensive equipment, dedicated laboratory space and highly trained laboratory technologists. Similarly, access to HIV nucleic acid detection for confirmation of early infant diagnosis of HIV infection is a challenge.
- Among these, the most promising is the use of dried blood spots (DBS). The process of DBS collection begins with a finger or heel prick and spotting whole blood directly onto filter paper, which is then left to dry at room temperature. Once dried, DBS can be stored with desiccant and shipped to central laboratories for HIV virologic testing. DBS has several advantages over traditional methods of sample collection: there is no need for phlebotomy; DBS increases the accessibility of HIV VL and EID testing in remote areas; it is a relatively easy and inexpensive means of collecting and storing samples under field conditions; DBS is easy to transport from peripheral site to central laboratory; and DBS reduces materials required, biological waste produced and sampling costs for specimen collection compared to venipuncture.
- Abacavir (ABC) is a NRTI recommended for the treatment of HIV-1 infection. It is a potent medication but has a limiting toxicity of hypersensitivity reaction (HSR), in 2–8% of treated patients. HSR to ABC is significantly associated with carriage of the HLA-B*57:01 allele and HLA-B*57:01 testing is recommended to be performed prior to ABC initiation.
- Some real-time PCR assays were developed for HLA-B*57:01 testing but not have been validated using whole blood blotted. We assessed the ability of a real time PCR to detect for the presence of HLA B*57:01 allele using DNA extracted from whole blood spotted on blotters.

MATERIALS AND METHODS

- A total of 200 genomic DNA samples were tested: 70 were HLA-B*57:01 allele positive and 130 HLA-B*57:01 allele negative. Whole blood belonged to HIV carriers, previously screened for HLA-B*57:01, was spotted on blotters and dry blood spots and stored at room temperature for 2 weeks before use.
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    - Martin et al, *Tissue Antigens*. 2005 Jun;65(6):571-4

RESULTS

- Our results show that our assay used from DNA extracted from dried blood spots was 100% sensitive, detecting all HLA-B*57:01 allele-positive patients and 100 % specific, having no false-positive results achieving a 100% specificity and 100% sensitivity on this control panel.

DISCUSSION AND CONCLUSIONS

- DBS samples have been used for many years for the diagnosis of infectious diseases, including the diagnosis of HIV. DBS have been shown to be an extremely useful tool in increasing the access to diagnostic tests, particularly in remote regions. In addition to the practical benefits of using DBS samples, the costs of sample collection and shipment are low compared to plasma samples. Whilst DBS samples appear to be useful for EIDHIV, VL and HLA B*57:01 allele measurements, concerns exist with respect to the assays and protocols.
- Dried blood spots are an alternative specimen type for HIV drug resistance genotyping and HIV-1 diagnosis in patients in resource-limited settings but can also be used to detect the presence of HLA B*57:01 allele.