APPLICATION OF RAPID DNA ISOLATION FOR ALPHA-1 ANTITRYPSIN GENOTYPING IN DRIED BLOOD SPOTS

R. Struniawski, A. Szpechcinski, B. Poplawska, M. Skronski, P. Kuca, Joanna Chorostowska-Wynimko

Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, 26 Plocka St., 01-138 Warsaw, Poland, email: j.chorostowska@igichp.edu.pl

Background: The use of dried blood spot (DBS) specimens for the genetic screening of α1-antitrypsin (AAT) deficiency is often limited by low quantity and quality of DNA extracted from DBS. The aim of our study was to present an optimized method of simple, fast and efficient DNA extraction from DBS samples using commercial kit that effectively eliminates any natural PCR inhibitors allowing for successful AAT genotyping by real-time PCR and direct sequencing. **Methods:** DNA extracted from 84 DBS samples from chronic obstructive pulmonary disease patients was genotyped for PI*S and PI*Z AAT deficiency variants by real-time PCR. The results of AAT genotyping were validated by IEF phenotyping and concentration measurement of AAT protein in sera from the same patients. The diagnosis of rare/unknown AAT variants was established by direct sequencing. **Results:** The proposed method of DNA extraction allowed successful DNA isolation from all analyzed DBS samples. Both quantity and quality of DNA were sufficient for further real-time PCR and genetic sequence analysis of all samples. The 100% concordance between AAT DBS genotypes and serum phenotypes in positive detection of two major deficiency alleles was achieved. Both assays, DBS AAT genotyping by real-time PCR and AAT phenotyping by IEF, positively identified PI*S and PI*Z allele in 8 out of 84 (9,5%) and 16 out of 84 (19%) patients, respectively. **Conclusion:** The procedure minimizes the hand-on-time of DBS samples preparation providing sufficient quantity and quality of genomic DNA to be used for either real-time PCR or genetic sequence analysis, as only good quality DNA template guarantees efficient and reproducible AAT genotyping results in any DBS screening protocol.