THE AGA KHAN UNIVERSITY HOSPITAL CLINICAL LABORATORIES

TEST UPDATE

Thalassemia testing by Beta Globin Gene Sequencing

Introduction

Beta-thalassemia is one of the most common genetic disorder in Pakistan. Variable clinical phenotypes of β-thalassemia arise from the presence of pathogenic mutations that affect the β- globin gene located on chromosome 11p15.5. So far, over 730 variants of the β-globin chain have been characterized and, more than 200 of those accounts for pathogenic mutations causing β-thalassemia. These sequence variants range from point mutations to large deletions and can affect either transcription or translation of the β-globin gene. The frequency and type of mutations occurring in a population may vary and, can be grouped into common and rare sets.

Allele-specific polymerase chain reaction (AS-PCR) is a common approach to assay for known β-globin gene variants, but it has limited sensitivity when using with a pre-defined panel of mutations. Mostly, AS-PCR assay cover only mutations commonly present in a population. Therefore, rare and unknown mutations can still go undetected. These methods are now being replaced by DNA sequencing technology.

Direct DNA sequencing-based analysis of the β-globin gene enables complete coverage and allows comprehensive identification of all possible variants (excluding large deletions) in a patient. Compared to allele-specific PCR, DNA sequencing can detect rare and unknown mutations at a higher sensitivity and in a shorter period of time

Principle of the Assay:

DNA is extracted from patient sample, followed by PCR amplification and bidirectional sequencing of the β-globin gene. This test covers all coding nucleotides, intron-exon boundaries, selected intronic, promoter and 3' untranslated regions.

Pathogenic mutations confirmed by DNA sequencing in a clinical sample are then associated to aphenotype using “Hemoglobin Variant Database” (a database of human hemoglobin variants andthalassemia mutations) at the globin gene server website.

Specimen Collection:

10 ml peripheral blood required in EDTA containing tubes.

Reporting Schedule:

Test is performed every Monday; reported after 10 working days