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abstract

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ADA-SCID Diagnosis: Development, Validation, and Clinical Application of an Adenosine Deaminase Activity Assay

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Introduction: Adenosine deaminase (ADA) deficiency, also known as severe combined immunodeficiency (SCID), is a rare genetic disorder caused by mutations in the ADA gene. Timely diagnosis is essential for initiating appropriate treatment and significantly impacting the prognosis of the disease. We modified the existing high-sensitivity HPLC (high-performance liquid chromatography) method for determining ADA activity in dried blood samples (DBS), which can be collected as part of routine newborn screening.

This test is based on enzymatic conversion of adenosine to inosine and hypoxanthine, followed by their quantification using HPLC. The objective of this study is to validate our method and establish a reference range for the early diagnosis of ADA-SCID.

Methodology: DBS samples were collected from 39 healthy children, 13 mutation carriers, and 11 confirmed ADA-SCID patients. The assay involved extracting enzymes from DBS, quantifying proteins, performing a hydrolysis reaction, and analyzing the samples using HPLC. ADA activity was measured in nmol/h/mg of protein.

Results: The method was validated for selectivity, linearity, lower limit of quantification, accuracy, precision, and sample stability. A reference interval for healthy donors of 14.94-40.39 nmol/h/mg was established. The mean ADA activity was 24.85 nmol/h/mg in the control group, 14.08 nmol/h/mg among carriers, and 2.18 nmol/h/mg in patients with ADA-SCID. Kolmogorov-Smirnov testing showed non-normal distributions in all groups.

The Mann-Whitney test showed significant differences between the control and carrier groups ($p=0.0003$), between the control and patient groups ($p<0.0001$), and between the carrier and patient groups ($p=0.0004$).

Conclusion: The developed HPLC method accurately determines ADA activity, effectively differentiating ADA-SCID patients from healthy individuals and heterozygous carriers. The reference interval, while useful for identifying ADA deficiency, does not provide sufficient information for carrier detection. The implementation of this method in clinical practice would improve early diagnosis and treatment monitoring for ADA-SCID.