ADENOSINE DEAMINASE ASSAY (ADA)

CE

Dual Vial Liquid Stable Assay

Convenient

- Stable liquid stable format requires no reagent preparation
- Lyophilized calibrator included with the kit *
- High and low controls available

Excellent Performance

- Excellent precision with CV's of less than 5%
- Extended linearity from 0 – 200 U/L
- Average recovery of 99.7%

Excellent Reagent Stability

- Three month on-board stability
- 12-month kit stability

Specific

- Highly specific for ADA and has no detectable reaction with other nucleosides
- Assay is not affected by serum bilirubin up to 30 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750 mg/dL, and ascorbic acid up to 4 mg/dL

Flexibility

- Requires as little as 5 µL ideal for pediatric, veterinary and research applications**
- Ability to test serum, plasma, CSF and pleural effusions **
- Automated and manual assay parameters available

*Packaged separately

**Assay is for research only in the United States.

INNOVATIONS IN CLINICAL DIAGNOSTICS

DIAZYME

ADENOSINE DEAMINASE ASSAY (ADA)

SUMMARY OF PERFORMANCE

Background

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, and is especially high in T lymphocytes. Elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma Increased ADA activity was also observed in patients with tuberculous effusions. Determination of ADA activity in patient serum may add unique values to the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests. ADA assay may also be useful in the diagnostics of tuberculous pleuritis.

Assay Method

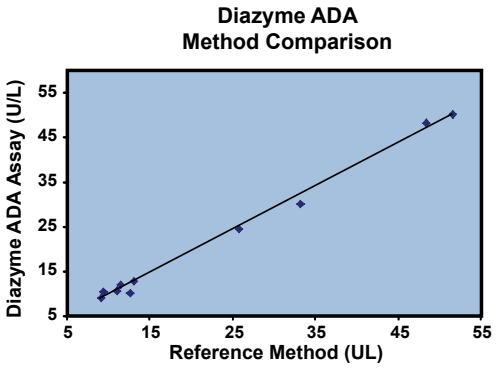
The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphory-lase (PNP). Hypoxanthine is then converted to uric acid and hydrogen perox-ide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.

Performance

Method Comparison

To demonstrate accuracy, the Diazyme Adenosine Deaminase Enzymatic Assay was tested with individual serum samples with comparison to results obtained by an accredited reference clinical laboratory using their analyte specific reagents based upon the reference method for ADA activity in serum.

The individual patient serum or plasma samples used for this study were from a certified commercial source. A small sample of ten (10) patient samples ranging from 13 - 48 U/L were tested which gave a correlation coefficient of 0.966. This study yielded a linear regression equation of y = 0.9662x - 0.02 U/L.



Precision

The precision of the Diazyme Adenosine Deaminase Enzymatic Assay was evaluated according to a modified Clinical and Laboratory Standards Institute (formerly NCCLS) EP5-A protocol. In the study, two specimens containing 11.0 ± 2.75 and 30.0 ± 5.4 U/L Adenosine Deaminase were tested with 2 runs per day with duplicates over 15 working days.

Within-run Precision	Level 1	Level 2
Mean (U/L)	11.11	30.74
SD	0.16	0.45
CV%	1.47	1.45

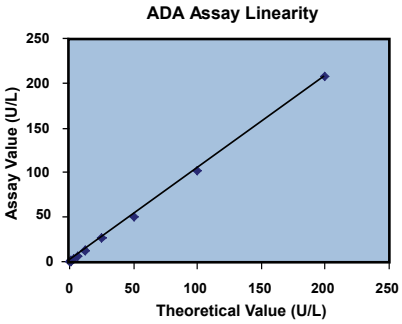
Between-Run Precision	Level 1	Level 2
Mean (U/L)	9.63	29.62
SD	0.47	0.59
CV%	4.90	2.00

Analytical Sensitivity

To demonstrate the limit of detection (LOD) of Diazyme Adenosine Deaminase Enzymatic Assay, Adenosine Deaminase zero calibrator was tested on 12 replicates on Cobas Mira. The LOD is defined as mean + 3SD. Based on these studies the LOD = 0.003 + 0.03 = 0.033 U/L Adenosine Deaminase.

Linearity

Ten levels of samples with ADA activity were prepared by serially diluting a serum control containing 200 U/L Adenosine Deaminase with distilled H_2O. Based on this study the assay is linear to 200 U/L.



Interference

To determine the level of interference from the substances normally present in the serum, Diazyme Adenosine Deaminase Enzymatic Assay was evaluated by running three (3) replicates each of a control sample in the absence and presence of various potential interference substances at indicated concentrations. Assay is not affected by interfering substances such as serum bilirubin up to 30 mg/dL, hemoglobin up to 500 mg/dL, triglycerides up to 500 mg/dL, ascorbic acid up to 20 mg/dL, and ammonia up to 800 µmol.

Interfering substances	Interfering substance concentration	Concentration of ADA (U/L)	Nonspiking (control)	% Interference
Ammonia	800 µmol	22.38 ± 0.10	22.76 ± 0.14	1.6
Ascorbic Acid	4.0 mg/dL	8.70 ± 0.17	9.20 ± 0.21	5.4
Bilirubin	30 mg/dL	41.0 ± 0.18	41.15 ± 0.19	2.6
Hemoglobin	200 mg/dL	123.0 ± 0.36	117.9 ± 0.16	4.2
Triglycerides	500 mg/dL	17.53 ± 0.20	18.05 ± 0.26	2.9

DIAZYME LABORATORIES

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