Audit Diagnostics

CE

ADA

Adenosine Deaminase

Colorimetric - Kinetic

Quantitative determination of Adenosine Deaminase (ADA) in serum and plasma samples
IVD

Store at 2-8ºC

PRINCIPLE OF THE METHOD

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulphopropyl)-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. The entire enzymatic reaction scheme is shown below.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

$$\text{Inosine} + Pi \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose 1-phosphate}$$

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye (max 556nm)}$$

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37ºC.

CLINICAL SIGNIFICANCE

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, 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 [1,2]. Increased ADA activity was also observed in patients with tuberculous effusions [3]. 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 [3].

REAGENTS

R 1	Tris-HCl pH 8.0	50 mM
	4-AA	2 mM
	PNP	0.1 U/mL
	XOD	0.2 U/mL
	Peroxidase	0.6 U/mL
R 2	Tris-HCl pH 4.0	50mM
	Adenosine	10 mM
	EHSPT	2 mM
ADA CAL		

PREPARATION

Reagents are ready to use. ADA Calibrator and Control are in lyophilized form, and need to be recontituted with 1.0 mL of distilled water before use.

PRECAUTIONS

R1 is light-sensitive and should be stored in a dark place.
Solution R1 and CAL contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
All specimens used in this test should be considered potentially infectious.

CALIBRATION

Recommend that this assay should be calibrated using the ADA Calibrator.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Stability: 1 month at 2-8ºC after opening, if contamination avoided and vials recapped immediately after use.
Signs of reagent deterioration:
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 540/550 nm.
- Thermostatic bath at 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Fresh serum and non-hemolyzed serum or plasma.
Stability: 7 days at 2-8ºC.

PROCEDURE

1. Assay conditions:
   Wavelength (main/sub): . . . . . .550 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature 37ºC
1. Mix 5 µl sample with 180 µl R1 and incubate at 37ºC for 3 minutes.
2. Add 90 µl R2 into cuvette, mix and wait for 5 minute.
3. Read initial absorbance and start timer simultaneously, read again after 3 minutes.
4. Calculate absorbance change per minute (ΔA/min)

CALCULATIONS

$$\text{ADA (U/L)} = \frac{\Delta A_{sample}/\text{min}}{\Delta A_{calibrator}/\text{min}} \times \text{Calibrator value}$$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures. ADA Control (2 levels).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES

0-15 U/L
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Linearity: The assay is linear up to ADA concentration of 200 U/L.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Precision: In the study, two serum specimens containing 11 and 30 U/L ADA were tested with 2 runs per day with duplicates over 15 working days:

	Within Run (N=30)	
	11 U/L	30 U/L
Mean (U/L)	11.11	30.74
SD	0.16	0.45
CV (%)	1.47	1.45

Run to Run (N=30)	
11 U/L	30 U/L
9.63	29.62
0.47	0.59
4.90	2.00

Sensitivity: The minimum detectable concentration of ADA with an acceptable level of precision was determined as 0 U/L.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

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) do not interfere.

NOTES

Audit Diagnostics has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. *Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 88: 266-271 (1993)*
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3. *Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnositic tool for tuberculous pleurisy. Thorax 50: 672-674 (1995)*

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