Adenosine Deaminase (ADA)
PNP-XOD / Kinetic Method

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. 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 diagnosis of tuberculous pleuritis.

TEST PRINCIPLE :
The ADA assay consists of four steps:
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.

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

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

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

$$H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 2H_2O + \text{Quinone Dye } (\lambda \text{ max } 546\text{nm})$$

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

REAGENTS COMPOSITION:

Active Ingredients	Concentration
Reagent 1	
Tris-HCl, pH 8.0	25 mMol/L
4-AA	4 mMol/L
PNP	0.3 U/mL
XOD	0.4 U/mL
Peroxidase	
Surfactant	0.1 U/mL
Reagent 2	
Tris-HCl, pH 7.0	25 mMol/L
Adenosine	11 mMol/L
EHSPT	4 mMol/L

ADA Controls:
Adenosine Deaminase (Bovine Liver) and BSA (Available Optionally)

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. (ADA Calibrator and Controls are available on request with extra cost).

Reagent Preparation:
Liquid two-reagent system, ready to use for both manual method and automated chemistry analyzers (Kinetics). ADA control and calibrator are also Liquid & stable till expiry at 2-8°C. Control and calibrator sold separately.

Reagent Stability and Storage:
Reagents are stable until their expiration date when stored at 2-8°C.

Specimen Collection and Handling:
Serum or Heparinized Plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant. Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. ADA content of blood is stable for 1 week when stored 2-4°C.

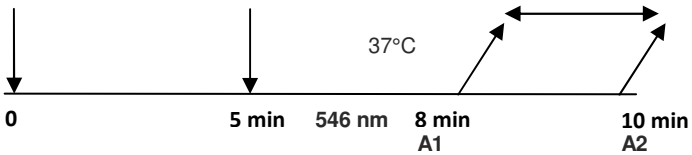
When the other body fluids (Pleural Fluid, Peritoneal Fluid, Cerebrospinal Fluid) are tested for ADA, ideal aseptic collection procedures should be followed.

Calibration:
Calibrator with a known valuevalue printed on the lables can be used to calibrate and validate the ADA assay.

Assay Procedure:

PROCEDURE FOR FULLY AUTOMATIC ANALYSER

R1 : 180 µL **R2 : 90 µL**
Calibrator/Sample : 5 µL 37°C



Quality Control:
Precision recommends that each laboratory should use ADA controls to validate the performance of ADA reagents. ADA Controls are available from Precision Biomed Pvt. Ltd.

Reference Range:
We have Tested ADA activity in 120 healthy human samples and the following reference ranges were drawn out of ADA assay.
- **Serum and Plasma samples were found to be in the range of 4-20 U/L**
- **Pleural Fluid values were found to be in the range of 0-40 U/L**
- **C.S.F. Values were found to be in the range of 0-5 U/L**

It is recommended that each laboratory should establish its own range of reference values.

Result interpretation:
Since Precision Adenosine Deaminase is intended for the determination of Adenosine Deaminase in various disease Conditions like Tuberculosis and Hepatic Disorders one has to clinically evaluate the disease condition before arriving at the diagnosis.

Linearity:
The Method is Liner up to 200 U/L.
Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

Interference:
Assay is not affected by serum bilirubin up to 31 mg/dl. Hemoglobin up to 220 mg/dl, Triglycerides up to 1000 mg/dl and ascorbic acid up to 4 mg/dl.

Analytical Sensitivity (Lower Detection Limit) : 4U/L.

Precision:
With – Run

N=20	level 1	Level 2
Mean(U/L)	31.53	35.45
SD (U/L)	0.17	0.17
CV (%)	0.54	0.48

Between – Run

N=20	level 1	Level 2
Mean(U/L)	32.20	35.59
SD (U/L)	0.95	0.67
CV (%)	2.95	1.90

Precaution:

1. Reagent R1 is Light-Sensitive. Store in a dark Place.
2. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
3. Avoid ingestion and contact with skin and eyes.
4. Do not use the reagents after the expiration date labeled on the outer box.

SYSTEM PARAMETERS FOR SEMI- AUTOMATIC ANALYSER

Reaction type	Kinetic Reaction (Increasing)
Wave length	546 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank /Zero Setting	With Distilled Water
Reagent Volume	R1 - 360 µl
	R2 - 180µl
Sample Volume	10 µl
Lag / Delay Time	300 Sec
Read Time	180 Sec.
Interval Time	60 Sec.
Linearity	200 U/l

Assay Procedure : For Serum / plasma / pieural / CSF / pericardial or, ascetic fluids

Reagent R1	360 µl
Serum/plasma/pleural/ CSF, pericardial or ascitic fluid	10 µl

Mix and incubate for 5 min at 37°C.

Reagent R2	180 µl

Mix and after 300 second incubation, measure the increase in absorbance every minute during 3 minutes at 37°C.

Pack Size :

Cat No.	Configuration	Pack
ADA00030	Reagent R1 - 1 x 20mL	30mL
	Reagent R2 - 1 x 10mL	
	Calibrator – 1 x 0.5mL	
ADA00090	Reagent R1 -3 x 20mL	90mL
	Reagent R2 - 3 x 10mL	
	Calibrator – 1 x 0.5mL	

References:

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. Am. J. Gastroenterol. 88: 266-271 (1993)

2. Kallkan A., Bult V., Erel O., Avci S., and Bingol N. K. : Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem Inst. Oswaldo Cruz 94(3) 383-386 (1999)

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)

IVD In Vitro Diagnostic Use | See Pack Insert For Procedure | Single Use only | CE

Temperature Limit | Manufacturer's Address | Manufacturing Date | Expiry Date | LOT Lot Number

Manufactured in India by :

Precision Biomed Pvt. Ltd.

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