ACCUCARE

Adenosine Deaminase(ADA)

PNP-XOD/Kinetic method

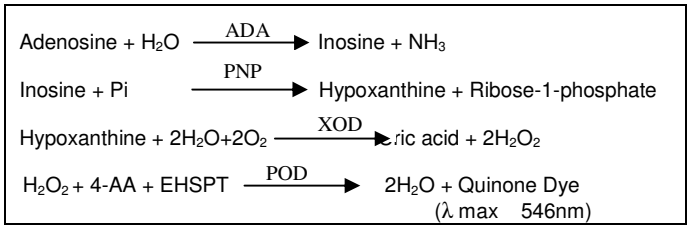
Quantitative determination of ADA activity in human Serum, plasma and other body fluids.
Only for *In Vitro* Diagnostic use

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 diagnosis of tuberculous pleuritis.

PRINCIPLE

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 (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline(TOOS) 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.



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

REAGENT COMPOSITION

Reagent 1	: Enzyme solution
Reagent 2	: Substrate solution
Calibrator	: 1 x 0.5 ml

SAMPLE COLLECTION AND PRESERVATION

Fresh serum and non-hemolyzed serum or heparinised plasma.
Stability: 7 days at 2-8ºC.
Ideal sample collection procedure to be followed when the other body fluids (Pleural Fluid, Peritoneal fluid, Perocardial fluid, Ascitic fluid and CSF) are tested for ADA.

REAGENT PREPARATION

The reagent is provided in a ready to use format.

REAGENT STORAGE AND STABILITY

Reagents are stable until the expiry date shown on the label when stored tightly closed at 2 – 8 ℃ and if contaminations are prevented during their use.

NORMAL VALUES

for serum, plasma Pleural paracardial and Ascetic Fluid

Normal	up to 43 U/L
Suspect for MTB	43 to 62 U/L
Strong Suspect for MTB	greater than 62 U/L

For CSF

Normal	up to 11 U/L
Suspect for TBM	11 to 12.35 U/L
Strong Suspect for TBM	greater than 12.35 U/L

MTB (Mycobacterium Tuberculosis)
TBM (Tuberculous Maningitis)

It is recommended that each laboratory establish its own references range to reflect the age, sex, diet and geographical location of the population.

AUTOMATED PARAMETERS	
Wavelength	546 nm
Measurement	Against DI Water
Reaction Temperature	37℃
Reaction Type	Kinetic
Reaction Direction	Increasing
Incubation	3 Min.
Sample Volume	10 µL
Reagent I Volume	400 µL
Reagent II Volume	200 µL
Delay/Lag/Time	300 Secs
Measuring Time	180 Secs
Interval Time	60 sec.
Linearity	200
Units	U/L

MANUAL ASSAY PROCEDURE

PIPETTE INTO TEST TUBES

REAGENT I	400 µl
SAMPLE	10 µl
Mix well and incubate for 3 mins at 37℃ & Immediately Add	
REAGENT II	200 µl
Mix Well, and read the change in absorbance (A0) after 6 mins (300 secs) & repeat the absorbance reading for every 1,2, & 3 mins(180) secs against D/W.Calculate the mean absorbance change/min.(ΔAbs/min).	

CALCULATION

$$\text{ADA activity (IU/L)} = \frac{\Delta\text{Abs/min Sample}}{\Delta\text{Abs/min Calibrator}} \times \text{Concentration of Calibrator}$$

LINEARITY

This method is linear upto a concentration of 200 IU/L.
Dilute samples above this concentration 1:1 with 0.9% saline and Repeat assay. Multiply the result by 2.

QUALITY CONTROL

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

BIBLIOGRAPHY

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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).

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