Adenosine Deaminase (ADA)-MTB
PNP-XOD / Kinetic Method

DIATEK

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 as well in the diagnosis of tuberculous pleuritis.

TEST 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 (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with TOOS and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.

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

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

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

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

REAGENTS COMPOSITION :

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

CODE No.	*ADA01*	*ADA02*
Pack size :	(30 ml)	(60 ml)
Reagent 1 (R1)	2 x 10 ml	2 x 20 ml
Reagent 2 (R2)	2 x 5 ml	2 x 10 ml
ADA Calibrator	1 x 1 ml	1 x 1 ml

(Conc. see on vial)

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8 ºC, protected from light and contaminations prevented.
Do not use reagents over the expiration date.
Consume the kit within 1 months after opening.

(ADA Controls are available on request with extra cost.
Use of assayed QC sera is recommended to validate test result.)

SAMPLES :

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

INTERFERENCES

Hemoglobin (up to 800 mg/dL), Triglycerides (up to 1000 mg/dL) and Ascorbic acid (up to 50 mg/dL) do not interfere.

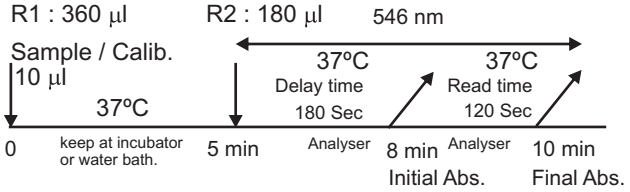
ASSAY CONDITIONS:

Wavelength : ..... 546 nm (540-555 nm)
Cuvette: ..... 1 cm light path
Constant temperature 37ºC
Reaction (Mode)..... Fixed Time
Number of Calibrator..... 1
Calibrator Conc ..... See on vial
Delay ..... 180 sec
Read time..... 120 sec
Linearity..... 176 U/L
Unit..... U/L
Slope of reaction..... Increasing

PROCEDURE :

1. Mix 10 µl calibrator / sample with 360 µl R1 and incubate at 37ºC for 5 minutes at incubator or water bath.
2. Add 180 µl R2, mix and read initial absorbance after 180 sec. and measure final absorbance after 120 sec. against distilled water blank at 546 nm by an Analyser.
3. Calculate absorbance change.

Assay Procedure summary:



CALCULATIONS :

$$\text{ADA (U/L)} = \frac{\Delta \text{ Abs of Test}}{\Delta \text{ Abs of Calibrator}} \times \text{Conc. of Calibrator}$$

REFERENCE RANGE :

SAMPLE	Status	Reference Range in IU/L
Serum, Plasma, Pleural Fluid, Pericardial fluid and Ascitic fluid	Normal	< 43
	Suspect for MTB	43 - 62
	Strong suspect for MTB	> 62
CSF	Normal	< 11
	Suspect for MTB	11 - 12.35
	Strong suspect for MTB	> 12.35

The above reference range is guideline and all the laboratories must establish their own normal reference range. This range can not be compared with the colorimetric (Glusti method) kit based on the estimation of ammonia released in the final reaction. Final diagnosis should be made with correlation of clinical factors.

LINEARITY AND DETECTION LIMIT :

The assay is linear up to ADA concentration of 176 U/L.
The minimum detectable concentration of ADA with an acceptable level of precision was determined as 4 U/L. The results of the performance characteristics depend on the analyzer used. 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.

BIBLIOGRAPHY:
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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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Medical Device Insert / Kit Insert / Instruction for use (IFU)
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