Enzymatic Kinetic Method

CODE: DL3201 - R1 - 1 X 10 ML + R2 - 1 X 5 ML DL3202 - R1 - 2 X 10 ML + R2 - 2 X 5 ML

INTENDED USE:

This reagent kit is intended for "in vitro" quantitative determination of Adenosine Deaminase (ADA) activity in serum and plasma samples and other body fluids.

CLINICAL SIGNIFICANCE:

Tuberculosis occurs worldwide. The most specific test is the positive bacterial culture of a patient's sample. This is cumbersome and time consuming. X-rays, smears for AFB and Tuberculin tests though comparatively rapid are not conclusive. Adenosine Deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T Lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. Though ADA is also increased in various infectious diseases like Infectious Mononucleosis, Typhoid, Viral Hepatitis, initial stages of HIV, and in case of malignant tumors, the same can be ruled out clinically.

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-3sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD) to generate quinine dye which is monitored in a kinetic manner.

Adenosine +
$$H_2O$$
 \xrightarrow{ADA} Inosine + NH_3 Inosine + Pi \xrightarrow{PNP} Hypoxanthine + Ribose-I-phosphate Hypoxanthine +2 H_2O + 2 O_2 \xrightarrow{XOD} Uric Acid + 2 H_2O_2 2 H_2O + 4-AAP + EHSPT \xrightarrow{POD} 2 H_2O + Quinone dye

REAGENT COMPOSITION:

Reagent 1: Enzyme Reagent Reagent 2: Substrate Reagent

MATERIALS REQUIRED BUT NOT PROVIDED:

- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:

Serum or heprinised plasma may be assayed. Venous blood should be collected and handeled anaerobically. Do not use citrate or oxalate as anticoagulant.

WORKING REAGENT PREPARATION & STABILITY:

Reagents are stable until their expiration date when stored at 2 -

GENERAL SYSTEM PARAMETERS:

Kinetic Reaction (Increasing) Reaction type

Wave length 546 nm Light Path 1Cm Reaction Temperature 37°C

With Distilled Water Blank / Zero Setting

Reagent Volume R1 - 360 µl R2 - 180_µl Sample Volume 10 µl

300 Sec. Lag / Delay Time Read Time 180 Sec. Interval Time 60 Sec. Factor 1743 200 U/I Linearity

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

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

Mix and incubate for 5 min at 37°C.

Reagent R2	180 μΙ

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

Determine the $\Delta A/min$.

CALCULATION: At 546 nm with 1cm Light path

ADA Activity in Serum / plasma / pleural / pericardial / CSF

or ascitic fluid (U/I) = Δ A/min. x 1743

LINEARITY:

Reagent is Linear up to 200 U/I.

Dilute the sample appropriately and re-assay if ADA Activity exceeds 200 U/I. Multiply result with dilution factor.

REFERENCE NORMAL VALUE:

For Serum, plasma, pieural, pericardial & ascetic fluids

up to 43 U/I Normal Suspect for MTB 43 U/I to 62 U/I Strong Suspect for MTB Greater than 62 U/I

For CSF

Normal Less Than 11 U/I Suspect for TBM 11 U/I to 12.35 U/I Strong Suspect for TBM Greater than 12.35 U/I

(Tuberculous Meningitis)

The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL:

For accuracy it is necessary to run known controls with every

BIBLIOGRAPHY:

- 1.Kobayashi F. Ikeda T. Marumo F. Sato C : Adenosine deaminase isoenzymes in liver desease. Am. J. Gastroenterol. 88: 266-271 (1993)
- 2.Kalkan A., Bult V., Erel O., Avci S., and Bingol N.K.: Adeonsine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem Inst. Oswaldo Cruz 94 (3) 383-386
- 3. Burgess L J, Matitz F J, Le Roux I, et al. Use of adenosine deaminase as a diagnostic tool for tuberculosis pleurisy. Thorax 50: 672-674 (1995)
- 4. Lakkana B., Sasisopin K: Use of adenosine deaminase for the diagnosis of tuberculosis : A review J. infect. Dis Antimicrob Agents 2010; 27: 111-8
- 5.Delacour H., Sauvanet C., Ceppa F., Burnat P.: Analytical Performances of the Diazyme ADA assay on the Cobas 6000 system. Clinical Biochemistry 43 (2010 1468-1471)

