FOR THE DETERMINATION OF ADENOSINE DEAMINASE ACTIVITY IN SERUM, PLASMA AND BIOLOGICAL FLUIDS BY ENZYMATIC METHOD

SUMMARY
Adenosine deaminase (ADA) is an endogenous tissue enzyme which is released into the serum in patients with different types of malignancies and infections, including viral hepatitis, infectious mononucleosis and tuberculosis. In pleural fluid elevated ADA levels are very commonly associated with tuberculosis. In CSF, ADA levels are elevated in cases of tuberculous meningitis. Increased concentration of serum ADA has shown the potential of usable screening test and can be used in the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests.

REAGENT
ADAZYME contains reagents for laboratory use only
ADAZYME comprises of:
  a) R1 - ADAZYME - Enzyme Reagent (Lyophilized).
  b) R2 - ADAZYME - Starter Reagent, ready to use.
  c) R3 - ADAZYME - Buffer Reagent, ready to use.
  d) C - ADAZYME - Calibrator (Lyophilized).

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 (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate a Quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction is as follows.

ADA – ENZYMATIC REACTION

\[
\begin{align*}
\text{ADA} & \quad \text{Adenosine} + \text{H}_2\text{O} \quad \rightarrow \quad \text{Inosine} + \text{NH}_3 \\
\text{Inosine} + \text{Pi} & \quad \xrightarrow{\text{PNP}} \quad \text{Hypoxanthine} + \text{Ribose-1-phosphate} \\
\text{Hypoxanthine} + \text{H}_2\text{O} + \text{O}_2 & \quad \xrightarrow{\text{XOD}} \quad \text{Uric acid} + \text{H}_2\text{O} \\
\text{H}_2\text{O}_2 + 4\text{-AA} + \text{EHSPT} & \quad \xrightarrow{\text{POD}} \quad \text{H}_2\text{O} + \text{Quinone dye } (\lambda_{\max} 546\text{nm})
\end{align*}
\]

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

REFERENCE RANGE
The ADA activities in 60 healthy human serum samples were found to be in the range of 0-15 U/L. For Pleural fluid values were found to be in the range of 0-24 U/L, for C.S.F. values were found to be in the range of 0-6 U/L, for pericardial fluid values were found to be < 40U/L and for Ascitic fluid < 30U/L. It is recommended that each laboratory establish its own range of reference values.

STORAGE AND STABILITY
1. Store the ADAZYME kit at 2-8°C, away from light.
2. Stability of the ADAZYME kit is as per the expiry date mentioned on the label.

NOTE
1. It is important that kit components from the same lot are used for achieving accurate and reproducible results. Do not intermix reagents from different lots.
2. The sequence of addition of reagents should be followed meticulously for achieving accurate results.

ADDITIONAL MATERIAL REQUIRED
Test tubes, test tube stand, waterbath/incubator (37°C), distilled or deionised water, variable volume pipettes, spectrophotometer with filter at 540-550 nm at 37°C or colorimeter with yellow filter, stopwatch.

REAGENT PREPARATION
ADAZYME is an enzymatic assay system that can be used for both manual method and automated chemistry analyser. The Enzyme Reagent (R1) is in (lyophilized) form; Reconstitute one vial of Enzyme Reagent with 10 ml of Buffer Reagent (R3) to prepare a Working Reagent. The Calibrator (C) is in (lyophilized) form; Reconstitute with 1 ml of distilled water. The Working Reagent is stable for 3 months at 2-8°C and reconstituted Calibrator is stable for 15 days at 2-8°C.

SPECIMEN COLLECTION AND PREPARATION
Cultured specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antibiotic drugs.

CSF: Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen.
Body fluids: Disinfect the site and collect specimen with aseptic precautions. Serum, Plasma: No special preparation of the patient is required prior to sample collection by approved techniques. It is recommended to use fresh sample for testing. Do not use haemolyzed, contaminated or turbid sample specimens.
ADA is reported to be stable in serum for 3 days at 2-8°C and in biological fluids for 2 days at 2-8°C, as after this, ammonia may be released in the samples even without any microbial contamination.

Assay Procedure
| Wavelength | 546nm |
| Temperature | 37°C |
| Light path  | 1 cm  |
For Manual Method

Pipeette into clean dry test tube as follows:

Addition sequence

| R1 | 0.400 ml |
| Test Sample/ Calibrator | 0.010 ml |
| Incubate at assay temperature (37 °C) for 3 minutes and add | |
| R2 | 0.100 ml |

Mix well and incubate for 5 minutes at 37 °C. Read the initial absorbance A1. Measure the change in absorbance per minute (ΔA/min) for the next 3 minutes.

Calculations

ADA Activity in U/L = ΔAT / ΔAC x Concentration of Calibrator

For Automated System

For Automated Clinical Chemistry analyzer, Programming/ System Parameters has to be adjusted as per the instrument setup.

Linearity

The linearity of the procedure is from 0 - 200 U/L.

Limitations

Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear, and if turbidity is seen then the reagent may have deteriorated.

If the sample ADA activity is greater than 200 U/L, the sample should be diluted with normal saline. The result should be multiplied by the dilution factor.

SAFETY PRECAUTIONS AND WARNINGS

(1) Reagent (R1) is light sensitive. Store in a dark place. (2) Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those Biosafety in Microbiological and Biomedical Laboratories (HHS publication Number [CDC] 93-8395). (3) As with any diagnostics test procedures, results should be interpreted considering all other test results and clinical status of the patient. (4) Avoid ingestion and contact with skin and eyes. (5) Do not use the reagents after the expiration date mentioned on the label.

System Parameters:

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<th>Reaction Type (Mode)</th>
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WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY