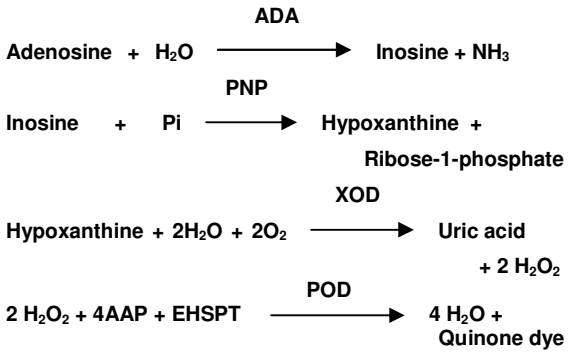
INTRODUCTION

1. ***Infinite*** ADA is a reagent set for direct quantitative determination of Adenosine deaminase (ADA) activity in human serum, plasma and other body fluids.
2. ***Infinite*** ADA is a ready-to-use, two liquid reagent system.
3. With ***Infinite*** ADA, the assay is linear upto 200 U/L.
4. ***Infinite*** ADA assay can be performed in 11 minutes at 37° C.

PRINCIPLE

ADA catalyses deamination of adenosine to inosine which is then 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-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4 AAP) in the presence of peroxidase (POD) to generate quinone dye.



The intensity of the colour developed is directly proportional to the activity of ADA in the specimen and is measured kinetically.

REAGENT STORAGE, STABILITY & HANDLING

The reagents R1 & R2 are ready-to-use.

The reagent kit should be stored at 2° - 8° C and is stable till the expiry date indicated on the label.

Reagent R1 is light-sensitive & to be stored in dark.

Contamination of the reagents should be strictly avoided.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
R1	
• Tris	>45mM
• PNP	>0.08 U/ml
• Xanthine oxidase	>0.15 U/ml
• Peroxidase	>0.40 U/ml
• Stabilizers, excipients & surface active agents	
R2	
• Tris	>40mM
• Adenosine	>8 mM
• EHSPT	>1mM

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Serum or heparinized plasma may be used. Do not use citrate or oxalate as anticoagulant. Ideally, venous blood should be collected & handled anaerobically.

It is recommended to use fresh sample. Serum or plasma, after prompt separation from cells or clot, should be kept tightly stoppered. Do not use hemolyzed, contaminated or turbid samples.

Ideal collection procedure, i.e. disinfecting the site & collecting with aseptic precautions, should be followed when other body fluids (Pleural fluid, pericardial fluid, ascitic fluid, cerebrospinal fluid) are tested.

ADA activity in serum/plasma is stable for 3 days when stored at 2° - 8° C & in other body fluids for 2 days when stored at 2° - 8° C .

Do not use specimens that are repeatedly subjected to freezing & thawing.

PROCEDURE

- **Reaction type……Kinetic**
- **Reaction direction……Increasing**
- **Wavelength……546 nm**
- **Flowcell temperature……37° C**
- **Zero setting with……Distilled water**
- **Delay time……300 seconds**
- **No. of readings……4**
- **Interval……60 seconds**
- **Sample volume……10 µl**
- **R 1 volume……360 µl**
- **R 2 volume……180 µl**
- **Factor……1743**
- **Linearity……200 U/L**

Manual assay procedure :

Perform the assay as given below:

R1	**0.360 ml (360 µl)**
Sample	**0.010 ml (10 µl)**
Mix & incubate for 3 minutes at 37° C.	
R2	**0.180 ml (180 µl)**

Mix & aspirate. After the initial delay of 300 seconds, record the absorbance of the test at an interval of 60 seconds for the next 180 seconds at 546 nm. Determine the mean change in absorbance per minute and calculate the test results.

Calculation :

Activity of ADA in U/L = ΔAbs/min. X 1743

EXPECTED VALUES

The indicative reference values for Indian population are as follows:

	Normal	Suspect	Strong suspect
Serum/plasma	< 22U/L	22 - 40U/L	> 40U/L
Body fluids (Pleural/Pericardial/Ascitic)	< 30U/L	30 - 40U/L	> 40U/L
CSF	< 9U/L	9 - 12 U/L	> 12U/L

Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own reference range representing its patient population.

NOTES

1. One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per minute at 37° C.

2. ADA activity in patients is elevated in different types of malignancies & infections like viral hepatitis, infectious mononucleosis, HIV and tuberculosis.

3. Hence, as with all diagnostic tests, it is recommended to interpret all the results in line with clinical manifestations, other test results & clinician's view collectively.

PROCEDURE LIMITATION

1. The reagent solution should be clear. Any turbidity indicates reagent deterioration.
2. If the ADA activity exceeds 200 U/L, dilute the specimen with normal saline (0.9% NaCl) and repeat the assay. The result obtained should be then multiplied with dilution factor to obtain correct ADA activity.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each laboratory should use a normal and an abnormal commercial reference control material. It should be realized that the use of quality control material checks both reagent and instrument functions together. Factors which might affect the performance of this test include instrument function, temperature control, cleanliness of glasswares and accuracy of pipetting.

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 diagnostic tool for tuberculous 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.*** 27:111-8 (2010).
5. Trivedi A., Chauhan K., Haridas N.: Diagnostic utility of Serum Adenosine deaminase and Liver Function Tests in Tuberculosis & HIV patients. ***Indian Journal of Applied Research.*** Vol 3: Issue 10 (2013).
6. Rao K. S.,Anand Kumar H., Rudresh B. M., et al.: A comparative study and evaluation of serum adenosine deaminase activity in diagnosis of pulmonary tuberculosis. ***Biomedical Research.*** 2: 189-194 (2010).
7. Kashyap R. S., Kainthla R. P., et al.: Cerebrospinal fluid adenosine deaminase activity: A complimentary tool in the early diagnosis of tuberculous meningitis. ***Cerebrospinal Fluid Research.*** 3:5 (2006).
8. In-house data: ***Accurex Biomedical Pvt. Ltd.*** (2015).

AR. No.: I107 LAD1-2015-07-001

Manufactured by:
Accurex Biomedical Pvt. Ltd.
Head Office – Mumbai. Tel.: 91(022) 67446744
E-mail:info@accurex.net ; Website: www.accurex.org
Plant : G-54, MIDC Tarapur, Boisar, Thane – 401 506. INDIA.
Call us toll free on: 1800 209 8456

Infinite
ADA
Enzymatic deamination