



ADA-MTB[®]

FOR THE DETERMINATION OF ADENOSINE DEAMINASE ACTIVITY IN SERUM, PLASMA & BIOLOGICAL FLUIDS

SUMMARY

Tuberculosis occurs worldwide and is rampant in many countries. Though curable, its infection is on the rise. The most specific test is the positive bacterial culture of a patient's sputum 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 cases of malignant tumours, the same can be ruled out clinically.

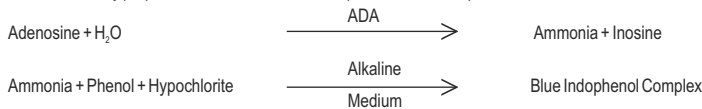
REAGENT

MICROXPRESS ADA-MTB is a reagent for laboratory use only.
ADA-MTB comprises of:

- L1** - ADA-MTB Reagent - Buffer Reagent, ready to use.
- L2** - ADA-MTB Reagent - Adenosine Reagent, ready to use.
- L3** - ADA-MTB Reagent - Phenol Reagent.
- L4** - ADA-MTB Reagent - Hypochlorite Reagent.
- S** - ADA-MTB Standard - ADA Standard, ready to use.

PRINCIPLE

Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.



REFERENCE VALUES

Serum, Plasma, Pleural, Pericardial & Ascitic Fluids	Normal	< 30 U/L
	Suspect	30 U/L to 40 U/L
	Strong Suspect	> 40 U/L to 60 U/L
	Positive	> 60 U/L
CSF	Normal	< 10 U/L
	Positive	> 10 U/L

It is recommended that each laboratory establish its own normal range representing its patient population.

REF	M-ADA015
▽	15

STORAGE AND STABILITY

- Store the ADA-MTB kit at 2°C-8°C, away from light.
- Stability of the ADA-MTB kit is as per the expiry date mentioned on the label.

NOTE

- 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.
- The sequence of addition of reagents should be followed meticulously for achieving accurate results.

ADDITIONAL MATERIAL REQUIRED

Test tubes, test tube stand, waterbath/incubator (35°C-37°C), distilled or deionised water, variable volume pipettes, spectrophotometer with filter at 570-630 nm (Hg 578 or 623 nm) at 37°C or colorimeter with yellow or red filter, stopwatch.

REAGENT PREPARATION

Reagents L1, L2 and standard are ready to use. **Adenosine Reagent (L2) may form crystals at 2°C - 8°C. Dissolve the same by gently warming (37°C - 50°C) the reagent for some time before use.** Both the Phenol Reagent (L3) & Hypochlorite Reagent (L4) need to be diluted 1:5 with distilled water before use (1 part of reagent + 4 parts of distilled water). The Working Phenol Reagent and Working Hypochlorite Reagent are stable for at least 6 months when stored at 2°C - 8°C in tightly closed bottles.

SPECIMEN COLLECTION AND PREPARATION

Collect specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antitubercular 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 specimen for testing. Do not use haemolyzed, contaminated or turbid sample specimens. Fresh EDTA, citrate, heparinised or oxalate anticoagulated plasma specimens are suitable for performing the test.

ADA is reported to be stable in serum for 3 days at 2°C-8°C and in biological fluids for 2 days at 2°C-8°C, as after this, ammonia may be released in the samples even without any microbial contamination.

TEST PROCEDURE

1. Bring all reagents and samples to room temperature before use.
2. Prepare the Working Phenol Reagent and Working Hypochlorite Reagent.
3. Set the spectrophotometer filter at 570-630 nm (Hg 578 or 623 nm) at 35°C-37°C.
4. Pipette into clean dry test tubes labeled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows

Addition Sequence	B	S	SB	T
	(mL)	(mL)	(mL)	(mL)
Buffer Reagent	0.20	0.20	-	-
Adenosine Reagent	-	-	0.20	0.20
Deionised water	0.02	-	-	-
Standard	-	0.02	-	-
Sample	-	-	-	0.02

5. Mix well and incubate at 37°C for exactly 60 minutes, and then add the following:

Working Phenol Reagent	1.00	1.00	1.00	1.00
Sample	-	-	0.02	-
Working Hypochlorite Reagent	1.00	1.00	1.00	1.00

6. Mix well and incubate at 37°C for 15 minutes or at R.T. for 30 minutes.
7. Measure the absorbance of the Blank (Abs. B), Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) against distilled water.

CALCULATIONS

$$\text{Total ADA activity in U/L} = \frac{\text{Abs. T} - \text{Abs. SB}}{\text{Abs. S} - \text{Abs. B}} \times 50$$

LINEARITY

The procedure is linear upto 150 U/L. If values exceed this limit dilute the sample with deionised water and repeat the assay. Calculate the value using the appropriate dilution factor.

PERFORMANCE CHARACTERISTICS

The ADA Standard provided in the above-mentioned product, corresponds to an ADA activity of 50 U/L when measured with the stated procedure. The ADA Standard has been standardized on the basis of CRM No. 647 i.e. Adenosine Deaminase (ADA-1) from the Community Bureau of Reference (BCR) of the European Commission.

REMARKS

(1) One unit of ADA activity releases three nanomoles of ammonia in the reaction in 1 hour at 37°C. (2) Patients with hyperamoniemia, kidney disorders and hepatitis can present high levels of ADA values. Patients with chronic malnutrition or HIV can present low levels of ADA values. (3) Higher levels of ADA are also found in leprosy, brucellosis, HIV infections, viral hepatitis, infectious mononucleosis and liver cirrhosis. Before arriving to a diagnostic decision, these clinical conditions must be ruled out. (4) Using a cut off level of 60 units/L of ADA, values have been reported to show the Specificity and the Sensitivity of the test as above 90% for the MTB infection. (5) Below 60 U/L of ADA, the serum ADA specificity and sensitivity is lower and should be interpreted in the light of other tests for confirmation of *Mycobacterium tuberculosis* infection.

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

(1) Jose, L., Banales, M.D., et. al. (1991), Chest 99/2 : 355. (2) Jose, M. Martinez-Vazquez, et. al. (1986), Gut 27 : 1049 - 1053. (3) Imma Ocana, et. al. (1986), Thorax 41 : 888 - 889. (4) Giusti, G., Galanti, B., Methods of Enzymatic Analysis, Pg. 1092 - 1099. (5) Lutwick, L.I., Tuberculosis, Chapman Hall Medical, 1st. Ed. Pg. 175. (6) Diagnostic Value of ADA and its Isoenzyme in Tuberculosis effusions, Dept. of Internal Medicine, Shiraz E- Medical Journal. (7) Data on file: Tulip Diagnostics (P) Ltd.

Temperature limitation	Manufacturer	Batch Number	Authorised Representative	Contains sufficient for tests	This way up	Phenol Reagent	ADA test for tuberculosis Test for the determination of Adenosine Deaminase Activity in serum, plasma and biological fluids
Catalogue Number	Consult Instructions for use	Use by	In vitro Diagnostic Medical Device	Date of Manufacture	Buffer Reagent	Hypochlorite Reagent	
					Adenosine Reagent	ADA Standard	



Manufactured by:

Microexpress

A Division of Tulip Diagnostics (P) Ltd.

Plot No. S-124, S-125, S-126, Utility Plot No. VIII, Phase III-B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain.

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