I-MYCOBACTERIA TEST (ADA TEST)

GQ-AT-50, GQ-AT-100

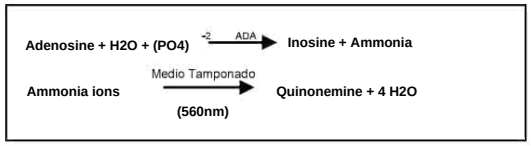
Colorimetric Method for the Determination of ADA in Serum and Biological Fluids

Clinical Significance

Serum adenosine deaminase (ADA) levels are used to assess the activity of pulmonary tuberculosis and have been identified as a marker of the response to conventional treatment for pulmonary TB. This product is RUO.
The most useful application of this technique is in the diagnosis of tuberculous meningitis and in the differential diagnosis between tuberculous and neoplastic pleurisy, since although ADA is also elevated in the pleural fluid of empyemas, rheumatoid arthritis and some lymphomas, the latter conditions are easier to differentiate clinically.

METHODOLOGY

The determination of ADA activity is based on the method of Giusti and Galanti; other methods based on Hillerbrand's method existed but resulted in a lower sensitivity of ADA in the diagnosis of tuberculosis.
The present method uses a modification of the Giusti and Galanti method, which is a calorimetric and enzymatic reaction.
Adenosine deaminase is the enzyme that catalyzes the reaction in an acid buffered medium, reacting with adenosine to produce the release of inosine and ammonia.
The ammonium ions formed in the presence of carbolic acid in an alkaline medium produce an intense blue color if the test is positive. The activity of the ADA enzyme was determined using Adenosine Deaminase (ADA) reagents. This is a calorimetric, enzymatic method based on the hydrolytic action of the ADA enzyme on adenosine to form inosine, producing a colored compound, quinoneimine, which is measured in the spectrophotometer at a wavelength of 560 nm.



COMPOSITION OF THE ADA BIALEX TEST REAGENT CONTAINS IN
100 mL Bottle 1: COLOR 1:
Carbolic
acid / Chloramine : 1g Na and K nitroferricyanide : 0.005g.
Bottle 2:
COLOR 2: Sodium/Hypochlorite: 5mL

Bottle 3:
Ada Test Standard (50U/L): Ammonium Sulfate Solution: 1.25 mL

Bottle 4:
Buffer pH 6.5: Sodium phosphate: 0.69454g.

Bottle 5:
Adenosine pa (CHNO / NaN) Adenosine: 2g

PRECAUTIONS

1. This reagent is for in vitro diagnostic use only.
2. The reagent is toxic; use gloves and a mask when handling the reagent.
3. If any spill occurs, wash the affected areas with plenty of water.
4. If a precipitate is present, place the reagent in a water bath at 50°C and homogenize until the precipitate dissolves.
5. It is recommended to use new tips for each reagent; combining these or reagent caps causes contamination.
6. They must be kept refrigerated at 2-8°C until their expiration date: COLOR 1, COLOR 2 and ADA TEST STANDARD

PREPARATION OF REAGENTS:
Ready to use

STABILITY AND STORAGE

- Reagents: COLOR 1, COLOR 2 and STANDARD: Must be stored at 2-8ºC - Reagents: BUFFER and ADENOSINE must be stored at room temperature.
- The reagents are stable until the expiration date indicated on the label

Do not use this reagent if:

- The Adenosine reagent is greater than 0.150 A
- When one of the reagents shows turbidity.
- It is reported by Bialex as a batch recall from the market.

COLLECTION AND CONSERVATION OF THE SAMPLE.

1. Samples for the ADA study are obtained through special procedures, in an aseptic manner, a condition that must be maintained until processing.
2. It is recommended not to work with a sample when it shows signs of hemolysis, as the presence of hemoglobin alters the results of the determination.
3. It is recommended to keep the sample at low temperatures (not frozen) until it reaches the laboratory. It should be refrigerated at 2-8°C.
4. It is then advisable to centrifuge at 1,500 rpm for 10 minutes, in order to remove any red blood cells, fibrin, mucus, etc.
5. If not processed immediately, freeze at -20ºC until processing.

INTERFERENCE

1. The following results were obtained in studies to determine the level of hemoglobin interference.

The presence of hemoglobin alters the results of the determination.
23. ADA is elevated in other non-tuberculous exudates, such as neoplastic effusions (mainly lymphomas, rheumatoid arthritis, adenocarcinomas, and mesotheliomas).

For diagnostic purposes, the results obtained in the test should always be evaluated together with the patient's medical history.

MATERIALS AND EQUIPMENT NOT INCLUDED

1. Clinical chemistry analyzer capable of measuring absorbance at 560 nm and equipment capable of maintaining a constant temperature of
2. 37 °C. Additional material required: pipettes and/or micropipettes, disposable tips, deionized water, stopwatch.

ASSAY PROCEDURE

Procedure for manual use For
optimal test performance, observe the instructions in this manual.
Note: If there is a precipitate in the Adenosine reagent, place the reagent in a 50ºC water bath until dissolved.

1. The reagent is ready to use. Leave the reagent at room temperature for a few minutes (those that are refrigerated).
2. Identify the test tubes, both for reagent blank, standard and sample blank and unknown samples.
3. Measure the indicated volumes.

	Blank	Standard	Sample Blank	Sample
Sample	-----	-----	-----	10µL
Adenosine	-----	-----	250µL	250µL
Bufffer 6.5	250µL	250µL	-----	-----
Standard	-----	10µL	-----	-----
Mix, cap the tubes, and incubate at 37°C for 60 minutes; then add:				
Color 1	500µL	500µL	500µL	500µL
Sample	-----	-----	10µL	-----
Color 2	500µL	500µL	500µL	500µL

4. Homogenize and incubate for 30 minutes at 37ºC.
5. Zero the spectrophotometer using a reagent blank.
6. Remove the tubes and read at 560nm, bringing to zero with distilled water.

CALCULATION

$$ADA\ (U/L) = \frac{\text{(Sample Absorbance Blank − Sample Absorbance)}}{\text{(Standard Absorbance Blank − Reagent Absorbance)}} \times \text{Standard Concentration}$$

Calculation Example:

$$\frac{(1.208 - 1.056)}{(0.259 - 0.062)} \times 50 = 38.5\ \text{U/L ADA in Aseptic Liquid}$$

Abs. Muestra : 1.208
Abs. Bl. Muestra: 1.056
Abs. Estándar : 0.259
Conc. Estándar : 50 U/L
Abs. Bl. Reactivo : 0.062

REFERENCE VALUES

Serum: 11 22 U/L
Cerebrospinal fluid: 0.9 U/L Pericardial fluid: 0.40 U/L Pleural fluid/serum Ratio Up to 1.3
Pleural Fluid: 0 35 U/L
Ascitic Fluid: 0 40 U/L
Synovial Fluid: 0 40 U/L

LINEARITY Up
to 250U/L of Ada. At higher linearity levels, dilute the sample with physiological saline and then multiply by the dilution performed.

SENSITIVITY
Studies conducted by Bialex demonstrate a sensitivity of 98%.

SPECIFICITY

Studies conducted by Bialex demonstrate a specificity of 99%.

QUALITY CONTROL.
The ADA test reagent has been evaluated on 50 serum samples from clinically normal patients, with results within reference limits, and on 20 serum samples from patients with tuberculosis, with 98% of the results being pathological. The same results were obtained for biological fluids (cerebrospinal and pleural fluid).

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Manufactured by Bialex®
717 Ohio Ave.
Wichita Falls, TX 76301
Website: http://www.bialexlab.com
E-mail: info@bialexlab.com

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