SUMMARY

The Adenosine Deaminase (ADA) is an enzyme of the purine salvage pathway; it catalyzes the hydrolysis of the adenosine to inosine. An increase of ADA activity in pleural fluid is useful in the assessment of tuberculous pleural effusion. For use with CSF see pag. 2.

PRINCIPLE

The Adenosine Deaminase (ADA) catalyzes the hydrolysis of the substrate Adenosine; the Ammonia obtained from this reaction, reacts with α-ketoglutarate and NADH by means of Glutamate dehydrogenase (GlDH) giving NAD.

The decrease of absorbance of NADH, for oxidation to NAD, is proportional to the activity of the ADA in the sample.

Components of the kit:

REAGENTS

- Good Buffer >20 mmol/L
- *REAGENT 1 (lyo) Adenosine >0.1 g/L
- *REAGENT 2 (lyo) NADH >0.25 mmol/L
- *REAGENT 3 (lyo) GlDH >1000 U/L
- Adenosine Deaminase (values in U/L met. UV at 37°C on the label)
- α-ketoglutarate >0,1 mmol/L
- Stabilizers

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date

AUXILIARY REAGENTS FOR CALIBRATION and for QUALITY CONTROL (Not supplied with the kit)

We suggest strongly to calibrate always on the instruments.

More Calibrator vials are available in the following kit:

- ADA CALIBRATOR Cod. ADACAL
- ADA CONTROL Cod. ADACO 1

PREPARATION OF THE WORKING REAGENT

*KIT 5 x 20 ml (Cod. ADA016)

Add 20 ml of *Reagent 1 (practically all the bottle) to one vial of *Reagent 2. Mix gently until dissolution.

STABILITY: 3 days at 2-8°C.

Mix kindly and let the working reagent reaches the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

PREPARATION OF CALIBRATOR

Add 1 ml of the distilled water to the *REAGENT 3 Calibrator (lyo); mix gently until complete solution. Avoid foam.

STABILITY: 5 days at 2-8°C in dark place.

3 months at -20°C, fractionated in small volumes.

CAUTION

The Calibrator is from human origin and has to be used as a potential transfer of infective pathologies. This product has been tested and found to be negative for HIV, HCV and HBsAg antibodies by an approved method. Because no test method can offer complete assurance that all infectious agents are absent, it is recommended that this product and all the samples be handled as though capable of transmitting infectious disease.

COMPONENTS OF THE KIT: 5 x 20 mL Cod. ADA016

For in vitro diagnostic use only

SAMPLE

- No haemolyzed fresh serum; No hemolyzed pleural liquid.

PROCEDURE

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against air or Distil. water
- Temperature: 37°C
- Method: kinetic
- Reaction: 3 minutes
- Linearity: till 150 U/L met. UV at 37°C

Let reagents reach the working temperature before using.

Pipette into a test tube or cuvette labelled:
S: Sample, ST: Calibrator/Standard :

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Reagent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>50 µl</td>
<td>---</td>
</tr>
<tr>
<td>Calibrator/Standard</td>
<td>---</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Mix carefully and incubate for 4 minutes at 37°C.

Measure the initial absorbances for Calibrator (Ast) and Sample (As).

Repeat the readings for at least 3 times at intervals of one minute.

Determine the average of the readings of absorbance/minute for Calibrator (ΔAst/min) and Sample (ΔAs/min).

CALCULATION

Insert the means found in the following formula:

Adenosine Deaminase (U/L 37°C) =

\[
\text{values of Calibrator} \times \frac{\Delta \text{As/min. Sample}}{\Delta \text{Ast/min. Calibrator}}
\]

REFERENCE VALUES

6,8 – 18,2 U/L  (UV method, 37°C)

It is advisable that every laboratory determine its normal reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: ADA may be determined between 3 - 150 U/L.

For concentrations ≥ 150 U/L, dilute the sample 1:4 with saline sol., repeat the determination and multiply the result x 4.

Sensitivity: The minimum detectable is 3 U/L.

Within-run Precision:

<table>
<thead>
<tr>
<th></th>
<th>Mean (U/L) ± 2s</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>8.2 ± 0.28</td>
<td>2.54</td>
</tr>
<tr>
<td>Serum 2</td>
<td>119.8 ± 6.9</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Run-to-run (Day-to-day) Precision:

<table>
<thead>
<tr>
<th></th>
<th>Mean (U/L) ± 2s</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>8.3 ± 0.38</td>
<td>2.92</td>
</tr>
<tr>
<td>Serum 2</td>
<td>120.6 ± 7.6</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Interferences: See References point 2.

Materlab S.L. Pº Pontones 7 28005 Madrid España tlf: 91/474 56 23 fax: 91/5175286 http://www.materlab.com materlab@materlab.com
Correlation: A group of 20 sera from 3 to 130 U/L was assayed by this procedure and using a similar commercially available ADA Reagent. Comparison of the data gave following results:

- Linear regression equation: \( y = 1.0332x + 0.43 \)
- Correlation coefficient: \( r = 0.9968 \)

**NOTE**
1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. Dilute the sample with activity higher than 150 U/L, with saline solution 1:4; repeat the determination and multiply the result by 4.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of ADA (see References 2).
5. PAY ATTENTION! Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.
8. Use sample fresh, as soon as available. DO NOT USE haemolyzed samples, because blood cells have very high concentration of ADA.

**REFERENCES**

**APPLICATION WITH CSF**

**REAGENTS, PREPARATION OF THE WORKING REAGENT**

- Reagents are the same of the kit, except for the Calibrator.
- To calibrate with CSF has to be used a particular ADA Calibrator for CSF (Cod. ADACSF).

**PREPARATION of ADA CALIBRATOR CSF**

- Dilute the content of one vial with 1 ml of distilled water (don't use saline or PBS, only dist. water).
- Mix kindly till complete dissolution.
- Please avoid foaming.
- A suggestion could be to aliquot in vials the quantity for each stage of analysis (a few days): to put the need for the day at 2-8°C for immediate use, to freeze the remaining vials for next days.
- Let reagents reach the working temperature before use.
- Close immediately after handling. Incompetent handling will release us from any responsibility.

**STABILITY:** the diluted *Calibrator is stable 1 day at 2-8°C in dark place.
- 2 months a -20°C, fractionated in small volumes.
- **FREEZE only ONE TIME.
- DO NOT REPEAT FREEZING.**

**CAUTION**

The Calibrator is from human origin and has to be used as a potential transfer of infective pathologies. This product has been tested and found to be negative for HIV, HCV and HBsAg antibodies by an approved method. Because no test method can offer complete assurance that all infectious agents are absent, it is recommended that this product and all the samples be handled as though capable of transmitting infectious disease.

**SAMPLE**

- No haemolyzed fresh CSF.

**PROCEDURE**

- **Wavelength:** 340 nm (334-365 nm)
- **Pathlength:** 1 cm
- **Reading:** against air or Distil. water
- **Temperature:** 37°C
- **Method:** kinetic
- **Reaction:** 5 minutes
- **Linearity:** till 30 U/L met. UV at 37°C
- **Sample/Reagent:** 1/4

Let reagents reach the working temperature before using.

**Procedure**

- Pipette into a test tube or cuvette labelled: S: Sample, ST: Calibrator/Standard :

<table>
<thead>
<tr>
<th>Working Reagent</th>
<th>S</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>250 µl</td>
<td>---</td>
</tr>
<tr>
<td>Calibrator/Standard</td>
<td>---</td>
<td>250 µl</td>
</tr>
</tbody>
</table>

- Mix carefully and incubate for 4 minutes at 37°C.
- Measure the initial absorbances for Calibrator (As1) and Sample (As2).
- Repeat the readings 5 minutes later for Calibrator (As2) and Sample (As2).
- Calculate the differences of absorbance/5 minutes for Calibrator \( \Delta As/5min = As1 - As2 \) and Sample \( \Delta As/5min = As1 - As2 \)

**CALCULATION**

Insert the values found in the following formula:

\( \text{Adenosine Deaminase (U/L 37°C)} = \frac{\Delta As/5min. \text{Sample}}{\Delta As/5min. \text{Calibrator}} \times \frac{\text{values of ADA Calibrator for CSF}}{\Delta As/5min. \text{Calibrator}} \)

**REFERENCE VALUES**

Not available Reference Values for CSF in literature.

It is advisable that every laboratory determine its normal reference values.

**ADDITIONAL NOTE with CSF**

1. For Calibration in automation we suggest to apply as follows:
   - CAL 1 --- Distilled water (conc. 0.0)
   - CAL 2 --- Calibrator for CSF (conc. mentioned in Cod. ADACSF)
2. We suggest don't dilute further the Calibrator for CSF.
   - If absolutely required, REM to dilute the Calibrator for CSF ONLY with distilled water.
   - USE IMMEDIATELY: stability at 2-8°C no more than half an hour.

Ver. 2006/06

Materlab S.L. Pº Pontones 7 28005 Madrid España tlf: 91/474 56 23 fax: 91/5175286
http://www.materlab.com materlab@materlab.com