a-PTT EA4

Activated Partial Thromboplastin Time (aPTT)
Ellagic Acid

CE

Code 1002/12 **Packaging 12x4 ml**

INTENDED USE

The Activated Partial Thromboplastin Time **(aPTT EA4)** is the most important method for monitoring the "intrinsic" pathway of coagulation of the blood and anticoagulant therapy with heparin. The ability of blood to form a fibrin clot by means of the intrinsic pathway requires phospholipids, calcium and a contact activator negatively charged, ellagic acid.
The **aPTT EA4** liquid Reagent is constituted by phospholipids from rabbit brain stabilized and optimized as a platelet substitute and a soluble plasmatic activator, ellagic acid, for an optimal activation of the contact phase of coagulation. The plasma sample is placed in contact with the Reagent containing an optimized amount of phospholipids and a contact activator negatively charged (R1); an incubation at 37°C for a defined time allows the activation of the intrinsic coagulation pathway. The addition of calcium ions (R2) to the reaction mixture starts the coagulation and it is determined the time required for the formation of the fibrin clot. The optimization of the aPTT liquid Reagent allows to have better or comparable performances to those one of the market leader Reagents, who employ ellagic acid: regardless that they use selected or synthetic or soybean phospholipids.

REAGENT COMPOSITION, CONTENTS and SAFETY WARNINGS

R1 - aPTT EA4 Reagent **5x4 ml**
Rabbit Brain phospholipids
Kaolin
Ellagic acid
Excipients and stabilizers
The Reagent is ready for use.
Before to use the container must be mixed by inversion several times to assure homogeneity of the Reagent. A lipid/ ellagic acid sediment might form upon prolonged standing of the containers.

R2 - CaCl2 **5x4ml**
CaCl2 >0.1 g/L
Excipients and stabilizers
The Reagent is ready for use.

NEVER FREEZE the Reagents.
Close immediately after handling. The Reagents have to be used correctly, to avoid contamination. An incompetent handling relieves us from any responsibility.
The product is not classified as hazardous pursuant to the provisions set forth in EC Regulation 1272/2008 (CLP) (and subsequent amendments and supplements).
Further information on the risks to health and / or the environment are given in the sect. 11 and 12 of MSDS.

STORAGE and STABILITY of REAGENT

The Reagents are stable up to the expiry date mentioned on the labels, stored at 2-8°C, if closed and kept in their intact primary container; if not exposed to heat sources and/or pressure variations.
In case of damaging of the primary container organize the waste disposal.

PREPARATION and STABILITY of WORKING SOLUTION

After opening the Reagents are stable for 30 days at 2-8°C in the original vial.
Stable for 5 days at 15°C on ACL systems (I.L) under mixing.
At the end of the working cycles is suggested to store the reagent to 2-8°C in the original vial for better stability.

SAFETY PRECAUTION

For in vitro diagnostic use only
Do not pipette by mouth
Exercise the normal precautions required for handling laboratory reagents

INSTRUCTIONS

A) The Reagent can be used with manual, mechanical, photometric and nephelometric clot detection systems. The automated determinations must be performed according to specific instructions attached to the instrument used.
B) Very deep attention must be given to interfering substances: certain drugs and other substances may influence levels of aPTT or aPTT assay (see References 2).
C) The Reagent must be used ONLY for the intended destinations, by expert and trained people and in according to good laboratory practice.
D) The clinical diagnosis cannot be done correctly using the result of only one test, but have to be done integrating critically the results of different laboratory tests and clinical data.
E) A series of factors, such as ambient temperature, the temperature of the working reagents, the accuracy of the washings, the type of coagulo-meter and the distilled water characteristics, can affect the performances of the test.

SAMPLES

- Collection of samples in accordance with CLSI (NCCLS) (see References 3) using citrated tubes.

PRECAUTIONS

All the precautions normally used in the laboratory must be respected for reagents handling. All the calibrators and controls must be considered as human sample, so potentially infectious; all the protection actions must be applied to avoid any potential biological risk.

ANALYTICAL PROCEDURE WITH MANUAL METHOD

- Preheat **R2 - CaCl2** at 37°C.
- Pipette into coagulation tube as follows:
- Plasma 100 µL
- **R1 - aPTT reagent** 100 µL
- Incubate for 5 minutes at 37°C.
- **R2 - CaCl2** 100 µL

Start the stopwatch at the same time the addition of the R2 - CaCl2. Determine the time of formation of the blood clot (seconds).

ATTENTION!

The kit is tested on an automatic coagulometer of ACL family (I.L.). Applications on automatic / semi-automatic or manual coagulometer from other manufacturers may be totally different from what we experienced.

CALCULATION and EXPRESSIONS OF THE RESULTS

Patient results can be expressed using the following units: seconds to form clots, clotting time ratio of patients versus the average of the normal clotting times.

REFERENCE VALUES

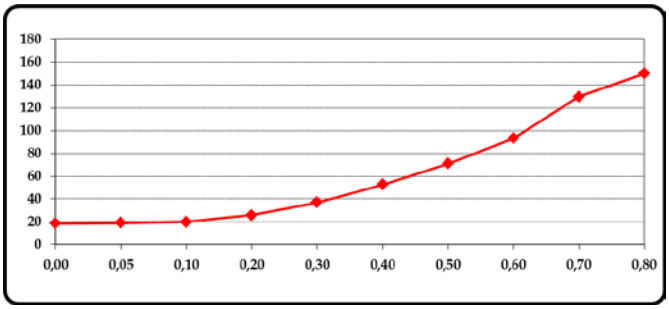
The results of aPTT are influenced by the method of determination of the clot, including Reagent and instrument, which may vary from laboratory to laboratory. Generally the aPTT test with normal plasmas originates clotting times between 19 and 31 seconds. However, each laboratory must establish its own normal range by testing individuals representative of the population.

ANALYTICAL PERFORMANCES
(validate on ACL7000 - ACL900 - ELITE PRO (I.L.))

The performances of the aPTT liquid Reagent have been tested with a ACL9000 Coagulometer. The data, while representing the characteristics of the product, could be different for each laboratory and for different coagulometers.

HEPARIN THERAPY:

Therapeutic range: 0.2-0.4U/mL.
We prepared solutions of normal plasma with scalar amounts of unfractionated Heparin to verify the sensitivity of the Reagent. The experimental results obtained in our laboratory with ACL9000 (I.L.), with specific lot of aPTT, are shown in this graph.



The results reported here are indicative and representative of the characteristics of the Reagent on our instruments, but in no case should be used as such in the individual laboratories. Since there are many variables that can affect results (eg. the use of different heparins), each laboratory should establish its own therapeutic reference range.

INTRINSIC FACTORS SENSITIVITY
The experimental tests have shown that the **a-PTT EA4** liquid
Reagent is sensitive to changes in the factors of the intrinsic pathway.

METTHOD LIMITATIONS: (see Bibliography 2)

INTERFERENCES
Interference test criterion: recovery ± 10% of initial value.
No interference found on samples with:

- total bilirubin up to 40 mg/dL;
- haemoglobin up to 600 mg/dL;
- lipemia [Intralipid ®] up to 2000 mg/dL;
- ascorbic acid up to 50 mg/dL.

WITHIN-RUN PRECISION
determined on 20 replications of 3 samples.
The results obtained are following:

Sample	Media (secondi) ± 2s	CV%
Human Plasma 1	19.45 ± 0.30	0.8
Human Plasma 2	29.80 ± 0.30	0.5
Human Plasma 3	36.03 ± 0.52	0.7

BETWEEN-RUN PRECISION
determined for 5 days on 20 replications of 3 samples.
The results obtained are following:

Sample	Media (secondi) ± 2s	CV%
Human Plasma 1	19.43 ± 0.27	0.7
Human Plasma 2	29.79 ± 0.76	1.3
Human Plasma 3	36.05 ± 0.92	1.3

ACCURACY
a group of 20 sera has been tested using this procedure and using a similar reagent available on the market. The comparison gave these results:

Linear regression y = 1.0032x + 0.054
Correlation coefficient r = 0.9933 n = 20

QUALITY CONTROL
To grant the right test performances use following kits (see the relative information for use (IFU)):

- **NORMAL CONTROL K29005301**
- **ABNORMAL CONTROL K29005302**

Each laboratory should establish its own range of control that determines the acceptable variation of the day-to-day performance for each control plasma.

BIBLIOGRAFY

1.Tietz Textbook of Clinical Chemistry, Ed. by C.A. Burtis, Elsevier Saunders Co., St. Louis-MO (2012).
2.Young D.S., Effect of drugs on Clinical Lab. Test, 5th Ed. AACC Press (2000).
3.CLSI(NCCLS) H21-A5: Collection, Handling, Transport and Storage for Hemostasis. Quick Guide.
4. CLSI(NCCLS) H47-A2: One-Stage PT Test and aPTT Test.
5. Hirsh, J. et al., Chest 108, 258S (1995).
6. Brill-Edwards P. et al., Ann. Int. Med. 119, 104 (1993).
7. Tripodi A. et al., Blood 104, 3631 (2004).

SIMBOLOGY

LEGENDA DEI SIMBOLI							
	Attenzione, consultare le istruzioni per l'uso			Test per kit			Produttore
	Unicamente per diagnosi in vitro			Scadenza			Monouso
	Conservare ad una temperatura compresa tra 2° e 30°C			Numero di lotto			Codice prodotto

PRODUTTORE

Meridian Healthcare srl
Via Caronda, 446 SC/A - 95129 Catania - Italy
Tel. +39 095 725 68 69 - Fax:. +39 095 725 44 54
info@meridianhealthcare.it
www.meridianhealthcare.it