

CALCIUM THROMBOPLASTIN

FREEZE-DRIED

CALCIUM THROMBOPLASTIN FOR THE PROTHROMBIN TIME DETERMINATION

6 x 4 ml

88 28 51

SUMMARY

Prothrombin or Quick time is the time, in seconds, necessary for a plasma to clot at 37° C, once a contained coagulation factor (thromboplastin) and CaCl₂ have been added. Abnormally long times with regard to those obtained for healthy people. Indicate a deficiency in some of the components of the coagulation system, while very short times are found in those patients with a tendency to form clots. Values obtained can be translated into percentages of coagulability or ratios of coagulability.

PRINCIPLE

The reagent is a calcium thromboplastin, freeze-dried, that allows the determination of variations in those coagulation factors (especially factors V, VII and X) that have been depressed during the treatment with coumarinic derivatives, in vitamin K deficiency, hepatopathy or due to a hereditary abnormality in the coagulation system.

REAGENTS

Contents:

6 x 4 ml Calcium Thromboplastin

The reagent has been obtained from a rabbit brain extract, and it is standardized from lot to lot.

STORAGE AND STABILITY

Unrehydrated vial is stable, when stored at 2° - 8° C, until the expiration date stated on the label. Once rehydrated will remain stable for a week at same temperature conditions. If necessary, the rehydrated reagent can be frozen, keeping its activity for no longer than 15 days. Avoid repeated freezing and thawing. Discard those vials with an evident contamination.

WORKING REAGENT

Rehydrate, a reagent vial with the volume of deionized water stated on the label. Mix gently. Prior to every determination, it is recommended to mix the solution to guarantee a complete homogenisation of the product. Once rehydrated, the solution contains:

Thromboplastin: Sufficient activity to promote the coagulation of plasma in the assay conditions.

CaCl₂: 0,01 m.

Stabilizers.

PLASMA PREPARATION

Mix carefully 9 parts of fresh blood with 1 part of trihydrated trisodium citrate at 3,8 % (0,13 m). Centrifuge at 3.000 rpm / 5 min and separate the supernatant plasma. Store in the refrigerator until prior to assay. (No longer than 2 hours).

PROCEDURE

1. Rehydrate a vial of reagent. Mix gently and bring to 37°C in a water bath for 15 min.
2. Dispense 0,1 ml of the plasma sample into a tube.
3. Incubate at 37°C/2 min.
4. Add 0,2 ml of calcium thromboplastin (at 37°C).
5. Start the stopwatch immediately and measure the time for the clot to be formed (Quick time).

The reagent can be used in those clot automatic detection devices. However, it has to be taken into account the special programming characteristics of every one, prior to assay.

RESULTS

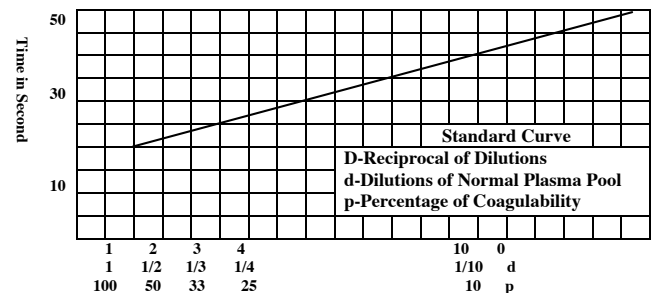
Results of the prothrombin time determination in one step have to be expressed in seconds or percentage of coagulability, that can be obtained in two different ways.

- A. From the measured time in seconds and, with the aid of the table, to find the percentage of coagulability.
- B. From the measured time in seconds and by interpolation in the calibration curve, to find the percentage of coagulability.

CALIBRATION CURVE

From a pool of citrated plasma, of at least five healthy male donors (18 - 40 years old), make the following dilutions:

Dilution	Not diluted	1/2	1/3	1/4	1/10
Citrated plasma	1	1	1	1	1
Saline	-	1	2	3	9
Percentage of coagulability	100%	50%	33%	25%	10%
Time in seconds (orientative)	10-12	13-15	17-19	20-22	40-45



USUAL VALUES

In percentage of coagulability: 80 - 100% for normal plasma samples. Therapeutic interval with oral anticoagulants is approximately of 20 - 35%.

LIMITATION OF THE PROCEDURE

Trace of detergent or blood in the glassware can give false results. Likewise, a wrong temperature in the water bath, unadequate proportions in the blood-citrate mixture or the use of aged samples whether for time or for temperature, will give rise to erroneous values in the determination.

Discard plasma samples from the ESR. In the case the venous puncture became arduous, the risk of an undesirable tissue thromboplastin aspiration is run.

REFERENCES

- Quick, A. J. (1975). Hemorrhagic diseases, Henry Kimpton, Londres.
 Hills, M., Ingram, I. C. (1973). Brit. J. Hematol., 25, 445-451.
 Heckemann, H. J. (1979). Thromb. Res., 15, 769-780