DIAGNOS THROMBO 1.0

Haemostasis Reagent for PT Determination

EXPLANATION OF THE TEST

DIAGNOS THROMBO reagent is a rabbit brain thromboplastin reagent for use in the *in vitro* testing of prothrombin times by photo-optical or mechanical clot detection systems. The test is used for the determination of the blood clotting factors II, V, VII and X (factor assays), for monitoring oral anticoagulant therapy, for diagnosing acquired or inherited bleeding disorders.

PRINCIPLE

DIAGNOS THROMBO is a rabbit brain thromboplastin reagent in liquid form, and an activator comprising calcium ions. The reagent is supplied as a liquid thromboplastin reagent with an activator containing calcium.

The method is responsive to depletion of the factors of the extrinsic system, including the vitamin K dependent clotting factors II, VII and X. Mixing Plasma with the reagent results ultimately in the formation of a clot. The time taken for the clot to form is measured and used to determine the anticoagulant status of the patient.

MATERIALS PROVIDED

1. DIAGNOS THROMBO Reagent (Ready to use with Preservative)
2. TSC Solution (3.2% Tri-sodium Citrate Solution)

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Pipettes for 100μl and 200μl
2. Waterbath at 37°C or other coagulometer
3. Stop Watch
4. Test Tubes

KIT PRESENTATION

2 ml

5 ml

STORAGE AND STABILITY

DIAGNOS THROMBO kit should be stored at 2-8°C in the coolest & driest area available. The kit has a shelf life of 24 months from the date of manufacturing.

Do not freeze the Reagent.

PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. For *in vitro* diagnostic use only.
2. Do not use the kit beyond the expiry date.
3. Do not eat or smoke while handling specimens.
4. Do not combine reagents from different batches as they are optimized for individual batch to give best results.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. The 37°C incubator should be calibrated daily since the Prothrombin time test functions accurately only at 37 $\pm$ 1°C.
7. The kit reagent, plasma should be properly pre-warmed at 37°C before use.
8. **ISI value is lot specific. The results should be calculated with given ISI only.**
9. **For best results, follow the given test procedure and storage instructions strictly.**

SAMPLE / SPECIMEN COLLECTION AND STORAGE

Collect 9 parts of venous whole blood in a clean container / tube having 1 part of TSC Solution (3.2% Tri-sodium Citrate solution) and immediately mix the blood with anticoagulant avoiding the foam formation. Centrifuge the sample for 15 minutes at approximately 2000g (3000 rpm) and collect the plasma in a separate tube. Fresh plasma is preferred for testing as it performs best when tested immediately after collection. However, if testing is delayed, sample may be tested within 2 hours at 25-30°C and within 3 hour at 2-8°C. Do not use haemolysed, lipaemic, turbid samples to avoid erroneous result.

TEST PROCEDURE

1. Always shake the **DIAGNOS THROMBO** reagent gently and properly before use.
2. Pipette 200μl of well mixed Diagnos Thrombo reagent into a test tube/ reaction cuvette.
3. Pipette 100μl of the patient plasma into a test tube / reaction cuvette.
4. Incubate the test tubes / cuvettes containing plasma and reagent in water bath (at 37°C) for 2-3 minutes.
5. Add 200μl of well mixed **DIAGNOS THROMBO** reagent pre-warmed to 37°C to the tube / cuvette containing plasma sample and simultaneously start the stopwatch. Tilt the test tube / cuvette back & forth and stop the stopwatch as soon as fibrin strand is visible which initiates gel clot formation.
6. Measure the time taken for clot formation to the nearest 0.1 seconds. This is the Prothrombin time (PT) in seconds.
7. Repeat steps 1-6 for the same sample and calculate the mean PT.
8. Record the patient INR using the ISI and MNPT supplied with the reagent.

For use with automated or semi-automated coagulometers, follow the manufacturer instructions.

CALCULATION OF RESULTS

The results may be interpreted directly in terms of PT of the test plasma in seconds (refer step 5 in test procedure) or as a ratio 'R'.

$$R = \frac{\text{Mean of the plasma PT in seconds}}{\text{MNPT of reagent}}$$

Or as International Normalised Ratio (INR), INR= $(R)^{ISI}$

where, ISI : "International Sensitivity Index" of the reagent.

(REFER INR CONVERSION TABLE).

ISI & MNPT for each lot is given with the kit.

It is recommended that each laboratory should establish its own MNPT by using plasma from 20 normal healthy individuals and then note their PT. Calculate the average time in seconds and it is MNPT.

THERAPEUTIC TARGETS

The following are the target INR recommended by the British Society for Haematology (Ref. 2) :

Target INR	Indication (Grade of recommendation)
2.5	Pulmonary embolus (A); Proximal deep vein thrombosis (A); Calf vein thrombus (A); Recurrence of venous thromboembolism when no longer or warfarin therapy (A); Symthromboomatic inherited thrombophilia (C); Non-rheumatic atrial fibrillation (A); Atrial fibrillationdue to rheumatic heart disease, congenital heart disease, thyrotoxicosis (C); Cardioversion (B); Mural thrombus (B); Cardiomyopathy (C)
3.5	Recurrence of venous thromboembolism whilst on warfarin therapy(C); Antiphospholipid Syndrome (B); Mechanical prostheticheart valve (B)

The Haemostasis and Thrombosis Task Force recommend that an INR within 0.5 INR units of the target is generally satisfactory.

The Expected Value for PT time is 11-16 seconds.

Oral Anticoagulant Therapeutic Range : INR = 2.0 - 3.5

SPECIFICITY AND CALIBRATION

DIAGNOS THROMBO reagent is sensitive to the concentration of the clotting factors II, V, VII and X in the test sample. The rabbit brain thromboplastin acts as a source of tissue factor and activates the clotting mechanism. Calcium is required to initiate clot formation.

Each batch of reagent is calibrated against a series of plasma samples which have assigned INR values for WHO Reference Thromboplastins rTF95 and OBT79.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCES

1. BCR No 148, Community Bureau of Reference, Brussels, Dec. 1st 1981.
2. Walker I D, Machin S, Baglin TP, Barrowcliffe T W, Colvin BT, Greaves M, Ludlam CA, Mackie IJ, Preston FE, Rose PE. Guidelines on oral anticoagulant: 3rd ed. British J. Haematol. 1998; 101:374-387.

INR CONVERSION TABLE

PATIENT R	ISI 1.00	ISI 1.05	ISI 1.10	ISI 1.15	ISI 1.20	ISI 1.25	ISI 1.30	ISI 1.35	ISI 1.40	ISI 1.45	ISI 1.50	ISI 1.55	ISI 1.60	ISI 1.65	ISI 1.70
1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1.1	1.10	1.11	1.11	1.12	1.12	1.13	1.13	1.14	1.14	1.15	1.15	1.16	1.16	1.17	1.18
1.2	1.20	1.21	1.22	1.23	1.24	1.26	1.27	1.28	1.29	1.30	1.31	1.33	1.34	1.35	1.36
1.3	1.30	1.32	1.33	1.35	1.37	1.39	1.41	1.43	1.44	1.46	1.48	1.50	1.52	1.54	1.56
1.4	1.40	1.42	1.45	1.47	1.50	1.52	1.55	1.57	1.60	1.63	1.66	1.68	1.71	1.74	1.77
1.5	1.50	1.53	1.56	1.59	1.63	1.66	1.69	1.73	1.76	1.80	1.84	1.87	1.91	1.95	1.99
1.6	1.60	1.64	1.68	1.72	1.76	1.80	1.84	1.89	1.93	1.98	2.02	1.07	2.12	2.17	2.22
1.7	1.70	1.75	1.79	1.84	1.89	1.94	1.99	2.05	2.10	2.16	2.22	2.28	2.34	2.40	2.46
1.8	1.80	1.85	1.91	1.97	2.02	2.08	2.15	2.21	2.28	2.35	2.41	2.49	2.56	2.64	2.72
1.9	1.90	1.96	2.03	2.09	2.16	2.23	2.30	2.38	2.46	2.54	2.62	2.70	2.79	2.88	2.98
2.0	2.00	2.07	2.14	2.22	2.30	2.38	2.46	2.55	2.64	2.73	2.83	2.93	3.03	3.14	3.25
2.1	2.10	2.18	2.26	2.35	2.44	2.53	2.62	2.72	2.83	2.93	3.04	3.16	3.28	3.40	3.53
2.2	2.20	2.29	2.38	2.48	2.58	2.68	2.79	2.90	3.02	3.14	3.26	3.39	3.53	3.67	3.82
2.3	2.30	2.40	2.50	2.61	2.72	2.83	2.95	3.08	3.21	3.35	3.49	3.64	3.79	3.95	4.12
2.4	2.40	2.51	2.62	2.74	2.86	2.99	3.12	3.26	3.41	3.56	3.72	3.88	4.06	4.24	4.43
2.5	2.50	2.62	2.74	2.87	3.00	3.14	3.29	3.45	3.61	3.78	3.95	4.14	4.33	4.54	4.75
2.6	2.60	2.73	2.86	3.00	3.15	3.30	3.46	3.63	3.81	4.00	4.19	4.40	4.61	4.84	5.08
2.7	2.70	2.84	2.98	3.13	3.29	3.46	3.64	3.82	4.02	4.22	4.44	4.66	4.90	5.15	5.41
2.8	2.80	2.95	3.10	3.27	3.44	3.62	3.81	4.01	4.23	4.45	4.69	4.93	5.19	5.47	5.76
2.9	2.90	3.06	3.23	3.40	3.59	3.78	3.99	4.21	4.44	4.68	4.94	5.21	5.41	5.79	6.11
3.0	3.00	3.17	3.35	3.54	3.74	3.95	4.17	4.41	4.66	4.92	5.20	5.49	5.80	6.13	6.47
3.1	3.10	3.28	3.47	3.67	3.89	4.11	4.35	4.61	4.87	5.16	5.46	5.78	6.11	6.47	6.84
3.2	3.20	3.39	3.59	3.81	4.04	4.28	4.54	4.81	5.10	5.40	5.72	6.07	6.43	6.82	7.22
3.3	3.30	3.50	3.72	3.95	4.19	4.45	4.72	5.01	5.32	5.65	5.99	6.36	6.75	7.17	7.61
3.4	3.40	3.61	3.84	4.09	4.34	4.62	4.91	5.22	5.55	5.90		6.66	7.09	7.53	8.01
3.5	3.50	3.73	3.97	4.22	4.50	4.79	5.10	5.43	5.78	6.15		6.97	7.42	7.90	8.41
3.6	3.60	3.84	4.09	4.36	4.65	4.96	5.29	5.64	6.01						
3.7	3.70	3.95	4.22	4.50	4.81	5.13	5.48	5.85							
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in vitro diagnostic reagent, not for medicinal use

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