



## THROMBIN TIME TEST FOR QUALITATIVE ESTIMATION OF FIBRINOGEN

### SUMMARY

At present there are known to be at least eleven factors in circulating blood, which are required for normal haemostasis. Deficiency in any of these factors viz. Factors I, II, V, VII, VIII, IX, X, XI and XIII results in a notable haemorrhagic condition, and the severity of the bleeding is proportional to the degree of deficiency. In order to treat the haemorrhagic condition, it is important to identify and quantify the deficient factor. FIBROSCREEN™ reagent is one such test reagent, which can identify the deficiency of Factor I (Fibrinogen). The reagent is used as a source of thrombin to determine the qualitative reactivity of fibrinogen.

### PRESENTATION

REF	10640061
Bovine Thrombin	6 x 1ml
Pack insert	1

### REAGENT

FIBROSCREEN™ reagent is a lyophilised preparation of bovine thrombin of ~ 50 NIH/ml. Reconstitute with the recommended volume (mentioned on the vial label) of distilled water, wait for 5 minutes, do not shake and mix gently by swirling till the solution attains homogeneity. Further keep aside for 10 minutes to attain equilibrium. Gently swirl the vial while drawing the reagent for use. Once reconstituted it is ready to use reagent for the thrombin time test.

### REAGENT STORAGE AND STABILITY

1. Store the unopened reagent vials at 2-8°C. **DO NOT FREEZE.**
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label and carton label.
3. **Once reconstituted the FIBROSCREEN™ reagent is stable for 6 days when stored at 2-8°C and for 4 hours at room temperature (20- 25°C), provided it is not contaminated. Extreme care has to be taken to maintain aseptic precautions while reconstituting, retrieving and handling reagents to prevent contamination. The FIBROSCREEN™ reagent vial must be replaced at 2-8°C immediately upon retrieving the reagent for the day's work.**

### PRINCIPLE

When a known quality and concentration of FIBROSCREEN™ reagent is added to citrated plasma, by observing the time required for clot formation and the quality of clot formed, a qualitative estimation of fibrinogen in the sample can be obtained.

### NOTE

(1) *In vitro* diagnostic reagent for laboratory and professional use. Not for medicinal use. (2) The reagent contains 0.01 % thimerosal as preservative. (3) FIBROSCREEN™ thrombin reagent is not from a human source hence contamination due to HBsAg, HCV and HIV is practically excluded. (4) As the bovine source is from non-BSE countries, the bovine source material included in this kit is considered to be free from risk for BSE/CJD and other and other zoonoses. However treat the material as if infectious. (5) It is very important that absolutely clean and dry micropipettes be used to aspirate and dispense the reagent. (6) Avoid exposure of the reagent to elevated temperatures, direct light and contamination. Immediately replace cap after use and store at recommended temperature. (7) Do not use damaged or leaking reagents.

### QUALITY CONTROL

A known normal control should be run in parallel with each batch of tests. This control may be TULIP plasma coagulation control PLASMATROL HI Cat. No. 11040061 or freshly drawn normal plasma.

### SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection by approved techniques. Withdraw blood without undue venous stasis and without frothing into a plastic syringe fitted with a short needle of 19 to 20 SWG. The venepuncture must be a 'clean' one and, if there is any difficulty, take a new syringe and needle and try another vein. Transfer the blood into tubes, after detaching the needle from the syringe. Mix exactly nine parts of freshly collected blood with one part of tri-sodium citrate (0.11 mol/l, 3.2%). Centrifuge immediately for fifteen minutes at 1500 g and transfer the plasma into a clean test tube. **Plasma must be tested within 3 hours of blood collection.**

### ADDITIONAL MATERIAL REQUIRED

10 x 75 mm glass test tubes, pipettes, Stop watch, distilled water, fresh plasma.

### PROCEDURE

Bring all the reagents and samples to room temperature before testing.

#### Manual method

Testing should be done in duplicate at room temperature. (20-25°C)

1. To a clean and dry 10 x 75 mm test tube add 200µl of plasma to be tested and 200µl of the reconstituted FIBROSCREEN reagent.
2. Start a stopwatch simultaneously with the addition of the FIBROSCREEN™ reagent.
3. Shake the tube gently to mix the contents and then tilt the tube back and forth.
4. Note the time **at the first appearance of the clot** and for the remaining portion of 60 seconds for consistency and character of the clot formed.

### INTERPRETATION OF RESULTS

Normal plasma begins to show clot formation within 15 seconds after FIBROSCREEN™ reagent has been added. **Because time of clot formation may be influenced by additional factors in the test system, estimation of approximate concentration of fibrinogen cannot be made from the initial clotting time alone but must be also made from observations of the consistency and character of the clot at 60 seconds.** At 60 seconds, samples with normal fibrinogen levels will form a firm clot that adheres to the walls of the test tube when the tube is

inverted. If either of these parameters is not met, (i.e. clotting time below 15 seconds or formation of a firm adhering clot after inversion of the test tube) **abnormality (less than 100mg%) of the fibrinogen reactivity should be suspected. In such cases quantitative estimation of fibrinogen using FIBROQUANT® Cat No 10641020 is strongly recommended.**

**EXPECTED VALUES**

A normal value using FIBROSCREEN™ reagent is the formation of a solid gel clot in 5-15 seconds, which adheres to the test tube wall on inversion at 60 seconds.

**REMARKS**

- FIBROSCREEN™ thrombin time remains normal in deficiencies of factor XIII (fibrin stabilizing factor).
- Fibrin gels may form in plasma with a fibrinogen concentration that is below normal. However, these gels are not firm, extrude considerable serum, and tend to slide on the side walls of the tilted test tube. Careful comparison of such gels with the firm clot with normal plasma used as a control will eliminate the possibility of confusion.
- FIBROSCREEN™ thrombin time test is usually performed first before any specific assays are attempted, when a prolongation of (PT and APTT) cannot be explained.

**Interpretation of first line tests:**

TEST				CONDITION
PT	APTT	TT	Platelet count	
N	N	N	N	Disorder of platelet function, factor XIII deficiency, disorder of vascular haemostasis, normal haemostasis
Long	N	N	N	Factor VII deficiency, Early oral anticoagulation
N	Long	N	N	Factor VIII: C, IX, XI, XII, Prekallikrein, HMWK deficiency, Von willebrand's disease, Circulating anticoagulant.
Long	Long	N	N	Vitamin K deficiency, Oral anticoagulants, factor V, VII, and II deficiency.
Long	Long	N	N	Heparin, Liver disease, Fibrinogen deficiency, hyperfibrinolysis
Long	Long	N	N	Thrombocytopenia
Long	Long	N	Low	Massive transfusion, Liver disease.
Long	Long	Long	Low	DIC, Acute liver disease.

N-Normal

**WARRANTY**

This product is designed to perform as described on the pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

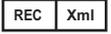
**PERFORMANCE CHARACTERISTICS**

FIBROSCREEN™ was evaluated with 30 samples having fibrinogen concentration between 100-400 mg/dl in comparison with other similar reagent. Data obtained using Fibroscreen reagent showed excellent correlation.

**BIBLIOGRAPHY**

(1) Biggs R. and R.G McFarlane: Human Blood coagulation and its disorders, Blackwell Scientific Publications, Oxford 1962. (2) Quick A.J: Hemorrhagic diseases and thrombosis, 2<sup>nd</sup> Edition, Philadelphia, Lea and Febiger, 1966. (3) CRC handbook Series in Clinical Laboratory Science, Section I: Haematology, Volume III, 1980. (4) Dacie J.V. and Lewis S.M.: Practical Haematology. 8<sup>th</sup> Edition, Churchill Livingstone Publications, 1995. (5) NCCLS guideline H21-A3, Vol. 18, No. 20. (6) Data on file: Tulip Diagnostics, India.

**SYMBOL KEYS**

	Temperature limitation		Manufacturer		This side up
	Use by		Consult Instructions for use		Authorised Representative in the European Community
	Date of Manufacture		Catalogue Number		Reconstitute with stated amount of distilled water
	Batch Number/ Lot Number		In vitro Diagnostic Medical Device		Description of reagent



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