BIOMEDICA DIAGNOSTICS

QuikCoag™ PT-HS

High-Sensitivity Prothrombin Time (PT-HS) Reagent

CE IVD For *In Vitro* Diagnostic Use

REF	Catalog Number	Quantity
	C.BMD.PTHS-02ML-8A	10 x 2mL
	C.BMD.PTHS-04ML-8A	10 x 4mL
	C.BMD.PTHS-10ML-20A	10 x 10mL

INTERNATIONAL SENSITIVITY INDEX (ISI)

Target ISI Value: 0.90 to 1.50

INTENDED USE

The **QuikCoag PT-HS** reagent is an *in vitro* diagnostic assay intended for use in performing the one stage prothrombin time (PT) test and assays which are based on a modified prothrombin time.

SUMMARY

The prothrombin time is the method of choice for monitoring oral anticoagulation therapy [1] and is a fundamental screening test for acquired or inherited bleeding disorders. During oral anti-coagulation therapy, the activity of vitamin K-dependant clotting factors (II, VII, IX, X, Protein C and Protein S) is reduced and PT time is increased. The test is used for quantitative determination of blood clotting factors in the extrinsic (VII) and common pathways (II, V and X) of coagulation [2,3].

PRINCIPLE

The capacity of blood to form a fibrin clot by way of the extrinsic hemostatic pathway requires thromboplastin, calcium, factors I, II, V, VII and X [4,5]. The **QuikCoag PT-HS** reagent provides a source of tissue thromboplastin and calcium that specifically activate factor VII in the extrinsic coagulation pathway. The factors involved in the intrinsic coagulation pathway are bypassed [6]. Therefore, deficiencies of intrinsic pathway factors (VIII, IX and XII) are not detected using the PT test.

REAGENT

The **QuikCoag PT-HS** reagent is a lyophilized preparation of rabbit brain thromboplastin, calcium chloride, buffer, and 0.05% sodium azide as a preservative. The lot number and expiry date of the reagent are shown on the vial's label.

PRECAUTIONS

Do not ingest. Avoid contact with skin, eyes or clothing.

REAGENT PREPARATION

1. Reconstitute the contents of the vial with the specified volume of purified water.
2. Replace the stopper and thoroughly mix the vial contents. Let stand for no less than 15 minutes prior to use to assure complete hydration of the contents.

STORAGE AND STABILITY

The reconstituted **QuikCoag PT-HS** reagent is stable for 5 days when stored in the original container at 2 to 8°C.

SPECIMEN COLLECTION AND PREPARATION

Test plasma should be prepared from citrated whole blood **without** heparin, EDTA or oxalate.

LIMITATIONS

1. Blood Collection using Syringe Method: Draw venous blood into a plastic or siliconized syringe. Immediately transfer 9.0 mL of blood into a tube containing 1.0 mL of 3.2% or 3.8% sodium citrate solution.

2. Blood Collection using an Evacuated Blood Collection Tube: Draw venous blood into a commercial vacuum tube containing 3.2% or 3.8% sodium citrate solution. Insure that a full draw has been obtained since the ratio of 9 parts blood to 1 part citrate is critical. A heparinized lock or transfer line should not be used. It is generally recommended that the second or third tube draw be used for coagulation tests.

3. Plasma Preparation: Mix well by inversion and centrifuge at 2,500 x g for 15 minutes soon after blood collection. Unless samples are to be processed immediately, transfer the plasma into a plastic tube. Plasma that is clearly hemolyzed or contains > 10,000 platelets per cubic milliliter or red cells is not suitable for coagulation testing. Plasma samples should be tested within two hours if maintained at room temperature.

4. Plasma Storage: For more details on specimen collection, handling and storage, please refer to CLSI Standard: Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition. CLSI Document H21-A5 (Vol. 28, No. 5), Wayne, PA, 2008.

PROCEDURE

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Pre-incubate the reconstituted **QuikCoag PT-HS** reagent to 37°C for at least 10 minutes. Maintain the suspension of the reagent by magnetic stirring or mixing by inversion immediately prior to use.
2. Pipette 100 µL of test or control plasma into a test cuvette
3. Incubate at 37°C for 1 minute.
4. Rapidly add 200 µL of the pre-incubated **QuikCoag PT-HS** reagent, simultaneously starting the timer.
5. Record the clotting time in seconds.

QUALITY CONTROL

Reliability of test results should be monitored within each run using normal and abnormal control plasmas such as the **QuikCoag Controls.** Each laboratory should establish a control range to determine the allowable variation in day-to-day performance of each control plasma.

CALCULATION OF RESULTS

Calculate the mean clotting time of duplicate samples and controls. Differences between duplicate results should be less than 5%. Repeat the test if necessary.

The PT result may be reported as seconds to form a clot, ratio of patient clotting time to mean normal clotting time, percent activity, or International Normalized Ratio (INR). The INR is recommended for use with patients undergoing anti-coagulation therapy.

INTERNATIONAL NORMALIZED RATIO

The International Committee for Standardization in Hematology and the International Committee on Thrombosis and Hemostasis have agreed on recommendations for the reporting of Prothrombin Time results as an International Normalized Ratio (INR). The INR is based on the International Sensitivity Index (ISI) of Thromboplastin reagents [7-9].

PT reagents are assigned an ISI value by calibration against an International Reference Preparation (IRP 67/40) with an assigned ISI of 1.0.

The INR is calculated using the following formula:

INR = (Patient PT / Mean Normal PT) $^{\textbf{ISI}}$

ISI = Lot specific International Sensitivity Index for the Reagent/Instrument system

Mean Normal PT = Lot specific mean of the normal range, as determined by each laboratory for the Reagent /Instrument System. It is usually based upon the mean PT plus or minus 2 to 3 standard deviations using 20 or more individuals.

To prevent spurious results ensure the blood to anticoagulant ratio is 9:1. The PT clotting times may be prolonged by substances including

corticosteroids, EDTA, oral contraceptives, asparaginase, clofibrate, erythromycin, ethanol, tetracycline and anticoagulants such as heparin and Coumadin. The PT may be shortened by substances including antihistamines, butabarbital, caffeine, oral contraceptives, phenobarbital and vitamin K [10].

EXPECTED VALUES

PT results are influenced by the method of clot detection and can vary from laboratory to laboratory. In general, PT tests performed with normal plasmas will give clotting times in the range of 10 to 16 seconds. However, each laboratory should establish a normal range using individuals representative of its patient population. A new normal range should be established with any change of instrumentation, blood collection techniques, or anticoagulant. A Mean Normal PT range should be reestablished or verified when changing lots of the same reagent [11].

Therapeutic ranges for monitoring oral anticoagulation therapy will vary from laboratory to laboratory. Therefore, it is essential that each laboratory establish relevant PT ranges for its respective patient population.

Abnormal results obtained with a plasma from a patient not on anticoagulant therapy may indicate a factor deficiency or the presence of an inhibitor. The result may also be due to the effects of certain drugs and medications [12]. Additional procedures such as an APTT and mixing studies using factor deficient plasma are usually required.

PERFORMANCE CHARACTERISTICS

Precision: Within-run precision was assessed using **QuikCoag Control Level 1 Normal, QuikCoag Control Level 2 Low Abnormal, and QuikCoag Control Level 3 High Abnormal**, on an optical and a mechanical instrument. The results are shown in the following table.

Within-run Precision Results

Sample	MLA™ Electra 900C (Optical)	Fibrometer (Mechanical)
Control Level 1 Normal	1.00 %	1.07 %
Control Level 2 Low Abnormal	0.31 %	1.48 %
Control Level 3 High Abnormal	0.36 %	1.08 %

Correlation: Correlation studies were performed against the PT-HS reagent of a competitor on the MLA™ Electra 900C coagulometer. The results are shown in the following table.

Correlation Results

Regression coefficient	Slope	Intercept
0.91	1.01	0.01

REFERENCES

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Symbols Key	
	Manufactured By
	Consult Instructions For Use
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Expiration Date (YYYY.MM)
	Temperature Limitations
REF	Catalogue Number
CON	Contents
REC	Reconstitution Volume
	Biological Risks
EC REP	European Authorized Representative