D-DIMER KIT

For use in semi-quantitative D-Dimer determinations
REF: K-707

For *in vitro* diagnostic use**.** **Store at 2°-8°C**

CE CORMAY

INTENDED USE

Latex agglutination test for semi-quantitative determination of fibrin D-dimer. D-dimer is formed by plasmin degradation of factor XIIIa cross-linked fibrin. Elevated levels of D-dimer are found in clinical conditions such a deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC).[1,2] D-dimer levels rise during pregnancy and high levels are associated with complications.[3] D-dimer Assay utilizes a monoclonal antibody specific for fibrin D-dimer but not for fibrinogen or fibrin.[2] D-dimer can therefore be determined in either plasma or serum samples.

CONTENTS AND PREPARATION

Product	D-Dimer Kit
Cat.No.	K-707
D-Dimer Latex	1 x 1.7 ml
Saline Solution	2 x 8 ml
D-Dimer Control (+)	1 x 1 ml
D-Dimer Control (-)	1 x 1 ml
Test cards	16pcs, 6 rings each
Mixing sticks	50pcs

Determinations: 80

1. D-Dimer Latex: 1 vial containing 1.7ml suspension of latex beads which are coated with anti-D-dimer monoclonal antibody and suspended in Hepes buffer, pH 8.2, with 0.2 g/l sodium azide as preservative. Before use, agitate the latex by repeatedly inverting the vial to disperse sedimented latex particles.
2. Saline Solution: 2 vials containing 8 ml each of buffered saline, pH 7.3. Contains 0.2 g/l sodium azide. Ready to use.
3. Positive Control Plasma: 1 vial containing 1ml lyophilized human plasma enriched with fibrin D-dimer. Contains 0.1 g/l merthiolate as preservative. Reconstitute with 1ml Saline Solution.
4. Negative Control Plasma: 1 vial containing 1ml lyophilized human plasma. Contains 0.1 g/l merthiolate as preservative. Reconstitute with 1 ml Saline Solution.
5. Test Cards: 16 test cards for 6 samples each.
6. Mixing sticks: 50 mixing sticks. May be broken into two pieces as desired.

Allow all vials to warm to room temperature before use.

Further materials required:

- Calibrated pipettes for 20, 50, 200 µl volumes
- pipette tips
- 3 ml test tubes
- Timer

STORAGE AND STABILITY

Reagents are stable until the expiration date shown on the label stored at 2° - 8°C. Reconstituted plasma:

	-20 ºC	2-8 ºC	20-25 ºC
D-Dimer Control (+)	3 months	1 month	4 hours
D-Dimer Control (-)	3 months	1 month	4 hours

PRECAUTIONS

Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV and HCV. However products from human blood should be considered as potentially infectious.
Some components of this kit contain sodium azide as a preservative. To prevent the build-up of potentially explosive metallic azides in metal plumbing, flush sinks with copious amounts of water and decontaminate regularly with sodium hydroxide solution.

SPECIMEN COLLECTION AND STORAGE

1. Obtain venous blood by clean vein puncture.
2. Immediately mix 9 parts blood with 1 part sodium citrate (3.2% or 3.8%) and mix well.
   (Note: Plasma anti-coagulated with citrate is recommended. EDTA anti-coagulated plasma or serum can also be used. Serum must be collected in the presence of fibrinolytic inhibitors (like in FDP tubes)).
3. Centrifuge the specimen at 1500g for 15 min. (platelet < 10000/µl).
4. Separate plasma after centrifugation and store in plastic or siliconised glass tube.
5. Use plasma within 4 hours, otherwise store frozen and thaw just prior to use.

Stability of plasma: 4h at 18-26°C 8h at 2-8° 14d at -20°C 6m at -70°C

PROCEDURE

A. Qualitative method

1. Place 20 µl of sample, positive and negative control plasmas in circles on a test card.
2. Place 20 µl of latex suspension in a nearby area of each circle.
3. Quickly mix the sample and latex using clean mixing sticks for each sample. Start the timer.
4. Rock the test card gently and read agglutination between 180 and 200 seconds. Positive (+) or negative (-) agglutination is compared to results obtained using the controls.

B. Semi-quantitative method meaningful only on samples tested positive.

1. Serially dilute 100 µl of sample 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 with 100 µl saline solution using small test tubes.

Mark the positions of sample dilutions on the test card and mix with latex suspension as Procedure A. The D-dimer concentration may be determined from the table in Results.

RESULTS

By testing serially diluted plasmas, semi-quantitative results can be obtained using the tables below. See Notes, 1.

D-dimer Level, ELISA scale, ng/ml

ng/ml	Undil.	1:2	1:4	1:8	1:16	1:32	1:64
< 250	-	-	-	-	-	-	-
250 -500	+	-	-	-	-	-	-
500 -1000	+	+	-	-	-	-	-
1000 -2000	+	+	+	-	-	-	-
2000 -4000	+	+	+	+	-	-	-
4000 -8000	+	+	+	+	+	-	-
8000-16000	+	+	+	+	+	+	-
> 16000	+	+	+	+	+	+	+

Plasma or serum containing more than 250ng/ml D-dimer gives an agglutination pattern represented by (+).

INTERPRETATION OF RESULTS

Agglutination occurs within 180-200 seconds for samples containing more than 250ng/ml (ELISA scale), therefore neat plasma or serum from normal, healthy individuals rarely agglutinates. If agglutination is observed within 180-200 seconds, a pathological condition probably exists. Note that the circulatory half-life of D-dimer is about 12 hours, so elevated D-dimer levels may persist for some time after the active process has ceased.

QUALITY CONTROL
The positive and negative controls provided in the kit should be used for quality control of the assay.

LIMITATIONS
1. Plasma containing rheumatoid factor may give false positive agglutination.
2. A negative D-dimer test does not completely rule out thrombosis. Detection of elevated levels of D-dimer should be used with other clinical information in forming a diagnosis. The negative predictive value for DVT has been reported to be 94% [5].

PERFORMANCE CHARACTERISTICS
In clinical studies on normal subjects, patients with phlebographically confirmed DVT, patients with DIC, and patients with pre-eclampsia (Pre-EC), the following results were obtained (See Notes 2):

Patient Group	Total #	# of patients with titre						
		Neg.	1:1	1:2	1:4	1:8	1:16	1:32 >1:64
Normal	101	100	1*	-	-	-	-	-
DVT	48	3	10*	7*	14*	6*	2*	4* 2*
DIC	29	0	3	3	4	2	6	3 8
Pre-EC	6	2	1	3	-	-	-	- -

* The agglutination was inhibited by addition of the D-dimer specific antibody (0.2mg/ml).

SPECIFICITY
The monoclonal antibody used in this device, MA-8D3, is specific for D-dimer by virtue of the screening method used for hybridoma selection[2]. No cross-reactivity with fibrinogen or des-AA-fibrinogen was observed when either analyte was substituted for plasma in this assay.
Plasma from 16 patients with rheumatoid arthritis was assayed and 14 were found to be non-agglutinating with the D-dimer Assay test. The agglutination could be inhibited by addition of the D-dimer specific monoclonal antibody MA-8D3 to these two samples, but not with addition of a monoclonal of the same subgroup, lgG_{1k} (PAM-1). This suggests that the positive responses in these two specimens were due to elevated D-dimer.

REPRODUCIBILITY
Three plasma samples were selected to test reproducibility of the assay. Each sample was tested 10 times on each of three different days. In each case, whereas positive results were obtained, the sample was titrated. The observed results were as follows:

Sample	D-dimer level	Result
Normal	<250 ng/ml	Always negative
Intermediate	3000 ng/ml	Titre always 1:8
High	> 4000 ng/ml	Titre always 1:64

ACCURACY
D-dimer Assay was compared to another commercially available latex assay using the "D-dimer Level, Latex Scale" table. Both products gave a negative reaction when tested on 25 normal specimens.

NOTES
1. The results may be reported using either the latex convention or the enzyme-linked immuno-adsorbent assay convention (ELISA). Some latex test results are expressed in fibrinogen equivalent units (FEU); 1ng/ml of D-dimer is about 2 FEU.
2. The diagnostic sensitivity of the D-dimer Assay for DVT, as calculated from the data above, is 94%.[5]
3. A small number of samples, when mixed with the latex, may exhibit white flakes which should not be confused with agglutination.
4. Agglutination will be more pronounced, and appears more rapidly, at higher D-dimer concentrations.

WARRANTY
This product is warranted to perform in accordance with its labelling and literature. P.Z. Cormay disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will P.Z. Cormay be liable for any consequential damages arising out of aforesaid express warranty.

REFERENCES

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