D-Dimer Latex Kit

IVD *For In-Vitro and professional use only*
Store at 2- 8°C

INTENDED USE

D-Dimer Latex Test is intended to be used to determine the cross-linked fibrin degradation products (XL-FDP) in human plasma using rapid qualitative and semi-quantitative detection.

INTRODUCTION

During blood coagulation, conversion of fibrinogen to fibrin occurs by the activation of thrombin. The resulting fibrin monomers polymerize to form a soluble gel of non-cross-linked fibrin. The fibrin gel is then converted to cross-linked fibrin to form a fibrin clot by thrombin activated Factor XIII. Formation of fibrin clot will trigger the production of major clot-lysing enzyme known as plasmin. The enzyme plasmin acts on both fibrinogen and fibrin which then will be cleaved to yield cross-linked fibrin degradation products (XL-FDP). The degradation products from cross-linked fibrin contain D-Dimer can be used as a specific marker of fibrinolysis detection.

PRINCIPLE

D-Dimer Latex is a rapid agglutination assay by observing the reaction of specific D-Dimer monoclonal antibody coated on latex particles. The test depend on the ability of XL-FDP present in human plasma to bind to the coated latex beads and agglutinate indicating that the concentration of D-Dimer in the tested sample is above the threshold of detection of the assay.

STORAGE CONDITIONS

The reagents should be stored refrigerated between 2°C to 8°C. **DO NOT FREEZE**. This reagent is stable until the expiry date stated on the product label. Do not use the reagents beyond expiration date. Failure of the latex reagent to agglutinate with the Positive Control, agglutination with the Negative Control, or evidence of microbial contamination are signs of reagent deterioration.

SPECIMEN COLLECTION AND PREPARATION

It is recommended to prepare plasma from whole blood anticoagulated with sodium citrate. An increased level of false positive reactions are noticed when using EDTA and heparin anticoagulated plasma. The specimen may be tested directly to detect the presence of XL-FDP after separation of the plasma by centrifugation (1500g for 15 minutes at 4°C - 10°C). It is not recommended to do defibrination of the plasma.
The plasma is stable when stored at -20ºC for 2 weeks. Make sure the frozen specimens are thawed rapidly at 37ºC and centrifuge before the testing.

MATERIALS

MATERIALS PROVIDED

- D-Dimer Latex Reagent: a 0.83% suspension of latex particles coated with murine anti-D-Dimer monoclonal antibody, 10mg/mL BSA and 0.1% sodium azide.
- D-Dimer Positive Control: a solution containing purified human D-Dimer fragment, 5mg/mL BSA and 0.1% sodium azide.
- D-Dimer Negative Control: a buffer solution containing 5mg/mL BSA and 0.1% sodium azide.
- Dilution Buffer
- Glass Reaction slide
- Stirring Sticks

MATERIALS NEEDED BUT NOT PROVIDED

- Precision pipettes and tips -20µL and 100µL
- Plastic test tubes and rack
- Stopwatch or timing device
- Disposable gloves
- Tissue (for wiping dropper bottle tips)

PROCEDURES

- The reagents should equilibrate to room temperature between 20°C to 25°C before use.
- Mix latex reagent by inversion immediately prior to use.

A. QUALITATIVE METHOD

1. Bring reagents and specimens to reach room temperature before use.
2. Place 1 drop (~20µL) of the latex reagent within a well on a reaction slide. Make sure not to touch the surface of the glass reaction slide.
3. Accurately pipette 20µL of undiluted plasma or of 1 drop of control solution inside the same well next to the drop of latex reagent.
4. Mix the latex reagent and sample by using a stirrer until the latex is uniformly distributed.
5. Rock the reaction slide gently by hand for exactly 3 minutes.
6. At exactly 3 minutes, check for agglutination under a strong light source.

NOTE: Test reading after more than 3 minutes may cause false agglutination pattern due to latex suspension may dry out. If this is suspected, the specimen must be retested.

B. SEMI QUANTITATIVE METHOD

1. Prepare serial dilutions of the test plasma with Buffer as follows:

1:2 dilution 100µL plasma plus 100µL Buffer solution
1:4 dilution 100µL 1:2 dilution plus 100µL Buffer solution
1:8 dilution 100µL 1:4 dilution plus 100µL Buffer solution

2. Test each dilution as described in the qualitative method.

READING AND INTEPRETATION

A. QUALITATIVE ASSAY

For the qualitative assay protocol, the following pattern of results should be obtained:

Undiluted Plasma Result	D-Dimer (XL-FDP) concentration
Negative	<0.20 mg/L (200ng/mL)
Positive	>0.20 mg/L (200ng/mL)

Note: All values in mg/L (ng/mL) are approximate

B. SEMI-QUANTITATIVE ASSAY

Approximate levels of XL-FDP, containing the D-Dimer domain, for specimen dilutions are shown in Table 1. As with all semi-quantitative tests, some variability in dose-response can be expected

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Approximate Range of D-Dimer (XL-FDP) mg/L (ng/ml)	Sample Dilution			
	Undiluted	1:2	1:4	1:8
< 0.2 (< 200)	-	-	-	-
0.2 – 0.4 (200 – 400)	+	-	-	-
0.4 – 0.8 (400 – 800)	+	+	-	-
0.8 – 1.6 (800 – 1600)	+	+	+	-
1.6 – 3.2* (1600 – 3200*)	+	+	+	+

"+" = agglutination, "-" = no agglutination
*** Levels of XL-FDP greater than 3.20 mg/L (3200 ng/mL) can be estimated by further dilutions beyond 1:8.**

INTERFERENCES

INTERFERING SUBSTANCES:

The presence of Rheumatoid Factor (RF): In a study from samples of patients with rheumatoid arthritis, 17 were found to agglutinate with D-Dimer latex. The agglutination could be inhibited by the addition of the D-Dimer specific monoclonal antibody DD3B6/22, but not with a non -specific monoclonal antibody of the same subgroup IgG3K. Thus, suggests that D-Dimer latex is insensitive to rheumatoid factor disturbances.

NONE INTERFERING SUBSTANCES:

- Hemoglobin 5.0 mg/mL
- Bilirubin 0.2 mg/mL
- Lipids (triglycerides) 30 mg/mL
- Protein (gamma globulin) 0.06 g/mL

EXPECTED VALUES

A positive result, indicating active fibrinolysis, should be obtained with D-Dimer Latex Test when XL-FDP (D-Dimer) levels are at or greater than approximately 0.20 mg/L (200ng/mL). Negative results means that plasma XL-FDP concentrations are typically less than 0.20 mg/L (200ng/mL). Each laboratory should establish its own normal range due to many variables that may affect results.
Elevated levels of XL-FDP contain D-Dimer has been showed in patients by a combination of immunoprecipitation and gel electrophoresis techniques. Monoclonal antibodies allow the specific detection of the D-Dimer domain. Monoclonal antibody based D-Dimer assay are vital for determination of conditions that are difficult to detect reliably by clinical examination such as diagnostic value in disseminated intravascular coagulation (DIC) and acute vascular diseases, including pulmonary embolism (PE) and deep venous thrombosis (DVT).

Several interrelated factors in vivo will helps to detect the amount of XL-FDP present in a specimen such as the severity of the thrombotic episode, the rate of cross linked fibrin formation, and the time elapsed after the thrombotic event until blood is drawn from the patient.
It has been reported that the elevated levels of XL-FDP can be as an indication of reactive fibrinolysis in surgery, trauma, sickle cell disease, liver disease, severe infection, sepsis, inflammation, and malignancy. During normal pregnancy, levels of D-Dimer increase but very high levels are associated with complications.

PROCEDURE LIMITATION

Diagnosis of diseases should include clinical sign along with the other relevant test information and must not only based on the results of D- Dimer Latex alone.

QUALITY CONTROL

Both Positive and Negative Controls should be included in each batch of tests to ensure proper functioning of the system. Make sure the control solutions are tested using the same procedures as patient samples.
D-Dimer Positive Control consists of a solution of human D-Dimer at a level of approximately ≥ 0.80 mg/L (≥800ng/mL).

PERFORMANCE CHARACTERISTICS

The sensitivity of D-Dimer latex test: 0.20 mg/L (200ng/mL).
In an anticoagulant study of 50 parallel citrated, EDTA and heparin plasma samples, the correlation between the titers obtained with D-Dimer Latex and the expected titer (based on ELISA XL-FDP values) as follows:

i. Citrated samples: r = 0.91
ii. EDTA samples: r = 0.73
iii. Heparin samples: r = 0.78

Citrate is the anticoagulant of choice.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- Protective clothing should be worn when handling the reagents.
- The reagents contain (0.1%) Sodium azide as preservative. Make sure to avoid any contact to skin or mucous membrane. The reagents contain sodium azide may be combined with copper and lead plumbing to form highly explosive metal azide. When drained, flush with a large volume of water to prevent azide build up.
- Use reagents as supplied and in accordance to the procedure mentioned. Do not use reagents past the expiration date.
- Do not use if the reagents found damaged or the label is not available.
- Discard the contents immediately if kit damaged or the glass vials are crack or leaking.
- Once the test is completed, wash hands and the test table top with water and soap.
- The procedure should be followed exactly as per the instructions in this package insert. Failure to do so may give false results or safety hazard.
- Close the vial tightly after each test.
- Don't drink or eat beside the reagents as it is considered toxic.
- Clean the spillage of reagent with disinfectant.
- The Positive Control in D-Dimer Latex Kit contain components of human origin. Each individual blood donation intended for the production of this reagent is tested for HBsAg, anti-HCV, anti-HIV1 and anti-HIV2. Only donations with negative findings are employed. As complete absence of infectious agents can never be assured, all materials derived from human blood should be treated as potentially infectious and handled with due care following the precautions recommended for biohazardous material.

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REF	Catalogue Number		Temperature limit		Manufacturer fax number		Fragile, handle with care
IVD	*In Vitro* diagnostic medical device		Caution		Manufacturer telephone number		Use-by date
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)		Keep away from sunlight		Date of Manufacture
LOT	Batch code		Manufacturer		Do not use if package is damaged		Keep dry