INTENDED USE

The DIMERTEST® Latex Assay is intended for the rapid qualitative or semi-quantitative evaluation of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human plasma.

SUMMARY AND EXPLANATION OF THE TEST

During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerize to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed.
Fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, but only degradation products from cross-linked fibrin contain D-dimer.[1-3] Therefore, cross-linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

TEST PRINCIPLE

DIMERTEST® Latex is a rapid agglutination assay utilizing latex beads coupled with a highly specific D-dimer monoclonal antibody. XL-FDP present in a plasma sample bind to the coated latex beads, which results in visible agglutination occuring when the concentration of D-dimer is above the threshold of detection of the assay.

REAGENTS

Composition

- R **Latex Reagent:** a 0.83% suspension of latex particles coated with murine anti-D-dimer monoclonal antibody, 10 mg/mL BSA and 0.1% sodium azide.
- P **Positive Control:** a solution containing purified human D-dimer fragment, 5 mg/mL BSA and 0.1% sodium azide.
- N **Negative Control:** a buffer solution containing 5 mg/mL BSA and 0.1% sodium azide.
- B **Buffer:** a 10 mM phosphate buffer solution containing 0.1% sodium azide.

Warnings and Precautions

- For In Vitro Diagnostic Use Only.

The Dimertest kit is classified as:

Warning
Hazard Class: Acute Tox. 4, H302
Hazard statements: H302: Harmful if swallowed.

Precautionary statements: P270: Do no eat, drink or smoke when using this product. P301+312: IF SWALLOWED : Call a poison center or doctor/physician if you feel unwell. P330: Rinse mouth.

- CAUTION: All reagents in DIMERTEST® Latex contain sodium azide (0.1%) as preservative. Do not ingest or allow to contact skin or mucous membranes. Sodium azide may form explosive azides in metal plumbing. Use proper disposal procedures.
- CAUTION: The Positive Control in DIMERTEST® Latex contains components of human origin. Each individual blood donation intended for the production of this reagent is tested for Hepatitis B antigen, 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.

Storage and Stability

Storage: Store at 2°C to 8°C. DO NOT FREEZE.
Stability: Refer to outer package and vial labels for expiration date.

Indication of Reagent Deterioration

Reagent deterioration is indicated by failure of the Latex Reagent to agglutinate with the Positive Control, agglutination with the Negative Control, or evidence of microbial contamination.

SPECIMEN COLLECTION AND PREPARATION

Plasma prepared from whole blood anticoagulated with sodium citrate is recommended. The use of EDTA and heparin will result in an increased level of false positive reactions. After separation of the plasma by centrifugation, specimens may be tested directly for the presence of XL-FDP. Defibrination of the plasma is not recommended. Plasma storage/stability:
-20°C: 2 weeks
Thaw frozen specimens rapidly at 37°C and centrifuge before testing. Refer to CLSI (formerly NCCLS) publication H21-A5 for further instructions on specimen collection, handling and storage.[4]

PROCEDURE

Materials Provided

DIMERTEST® (Cat. No. 49738960)

Latex Reagent	1 x 2 mL: White Cap	**Negative Control**	1 x 0.6 mL: Black Cap
Positive Control	1 x 0.6 mL: Yellow Cap	**Buffer**	1 x 20 mL
Test Cards	x 10: 8-well, for agglutination reaction		
Stirrers	x 60: for mixing		
Instructions for Use			

Materials Required but not Provided

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

Important!

- Equilibrate reagents to room temperature (20°C to 25°C) before use.
- Latex Reagent should be mixed by inversion immediately prior to use.
- Prior to each use, the dropper bottle tips must be wiped dry with a tissue.
- Dropper bottles must be held vertically when dispensing drops of reagent.

For correct drop delivery

Wipe tip dry prior to use

Qualitative Method

1. Mark (or make note of) positions on the test slide for specimens and, as needed, for positive and negative controls.
2. Hold the Latex Reagent dropper bottle vertically and place one drop of the reagent within a well on a Test Card.
3. AVOID touching the surface of the Test Card. Accurately pipette 20 µL of undiluted plasma or add one drop of control solution inside the same well next to the drop of Latex Reagent.
4. Mix the Latex Reagent and sample with a stirrer until the Latex is uniformly distributed.
5. Rock the Test Card gently by hand for exactly 3 minutes.
6. At exactly 3 minutes, check for agglutination under a strong light source. Note: If test reading is delayed beyond 3 minutes, the latex suspension may dry out **giving a false agglutination pattern. If this is suspected, the specimen must be retested.**
7. Discard the Test Card and stirrer into a biohazard container – do not re-use.

Semiquantitative Method

1. Prepare serial dilutions of the test plasma with Buffer as follows:
   1:2 dilution 100 µL plasma plus 100 µL Buffer solution
   1:4 dilution 100 µL 1:2 dilution plus 100 µL Buffer solution
   1:8 dilution 100 µL 1:4 dilution plus 100 µL Buffer solution
2. Test each dilution as described in the qualitative method.

QUALITY CONTROL

It is recommended that both Positive and Negative Controls be included in each batch of tests to ensure proper functioning of the system. Control solutions should be tested by the same procedures as patient samples. For the qualitative screening procedure, a positive result (agglutination) can be obtained by substituting DIMERTEST® Positive Control for the plasma in steps 3-6. Conversely a negative result (no agglutination) can be obtained by substituting DIMERTEST® Negative Control for the plasma in steps 3-6. When performing the semiquantitative procedure, it is recommended that testing of serial dilutions of the Positive Control be conducted. Dimertest® Latex Positive Control consists of a solution of human D-dimer at a level of approximately 0.80 mg/L (800 ng/mL).

RESULTS

A. Qualitative Assay

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

Undiluted Plasma D-dimer	(XL-FDP) concentration
Negative	Less than 0.20 mg/L (200 ng/mL)
Positive	Greater than 0.20 mg/L (200 ng/mL)

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

B. Semiquantitative Assay

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

Table 1

Approximate Range D-dimer (XL- FDP) mg/L (ng/mL)		Sample Dilution Undiluted	1:2	1:4	1:8
<0.20	(<200)	-	-	-	-
0.20 - 0.40	(200 - 400)	+	-	-	-
0.40 -0.80	(400 - 800)	+	+	-	-
0.80 - 1.60	(800 - 1600)	+	+	+	-
1.60 - 3.20*	(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.

LIMITATIONS OF THE PROCEDURE

Clinical diagnosis should not be based on the result of DIMERTEST® Latex alone. Clinical signs and other relevant test information should be included in the diagnostic decision.

EXPECTED VALUES

A positive result, indicating active fibrinolysis, should be obtained with DIMERTEST® Latex when XL-FDP (D-dimer) lev are at or greater than approximately 0.20 mg/L (200 ng/mL). Plasma specimens from normal subjects are expected to give negative results because their plasma XL-FDP concentrations are typically less than 0.20 mg/L (200 ng/mL). Due t many variables that may affect results, each laboratory should establish its own normal range. Elevated levels of XL-FDI (containing the D-dimer domain) have been demonstrated in patients by a combination of immunoprecipitation and gel electrophoresis techniques.[1-3,5,6] Monoclonal antibodies allow the specific detection of the D-dimer domain.7 Monoclona antibody based D-dimer assays are of diagnostic value in disseminated intravascular coagulation (DIC) and acute vascular diseases, including pulmonary embolism (PE) and deep venous thrombosis (DVT), conditions that are difficult detect reliably by clinical examination.[8,9]
The amount of XL-FDP detected in a specimen will depend on several interrelated factors in vivo, such as the severity o the thrombotic episode, the rate of cross linked fibrin formation, and the time elapsed after the thrombotic event until blo is drawn from the patient.
Elevated levels of XL-FDP as an indication of reactive fibrinolysis have also been reported in surgery, trauma, sickle cell disease, liver disease, severe infection, sepsis, inflammation, and malignancy.[10,11] D-dimer levels also rise during norma pregnancy but very high levels are associated with complications.[12]
DIMERTEST® Latex does not cross-react with fibrinogen, factor XIIIa cross-linked fibrinogen,[13] or fibrinogen degradatio products.[6]

SPECIFIC PERFORMANCE CHARACTERISTICS

Plasma from one hundred and seventy (170) apparently healthy, voluntary blood donors was tested using DIMERTEST Latex. A negative result was obtained for one hundred and sixty-two (162) of the samples. This equates to a specificity c 95.3% (162/170).
One hundred and forty-five (145) plasma samples from patients judged to be suffering from, or having a high probability for thrombotic episode, were tested by DIMERTEST® Latex and another agglutination reference method. The correlatio coefficient was r = 0.94 and the regression equation was y = 1.19x.
Intra-assay (within run) reproducibility was determined for 10 replicates of 3 plasma samples that contained different lev of XL-FDP. The results were equivalent for all replicates.
Inter-assay (run-to-run) reproducibility was determined using 10 plasma samples with XL-FDP titers ranging from 1 to 1 In 10 runs, the replicates of these specimens did not vary by more than one titer.
In an anticoagulant study of 50 parallel citrated, EDTA and heparin plasma samples, the correlation between the titers obtained with DIMERTEST® Latex and the expected titers (based on ELISA XL-FDP values) was r = 0.91 for citrated samples, r = 0.73 for EDTA samples and r = 0.78 for heparin samples. Citrate is the anticoagulant of choice. In a study c samples from patients with rheumatoid arthritis, 17 were found to agglutinate with DIMERTEST® Latex. In all 17 sample the agglutination could be inhibited by the addition of the D-dimer specific monoclonal antibody DD3B6/22, but not with non-specific monoclonal antibody of the same subgroup, IgG_{2b}. This suggests that DIMERTEST® Latex is insensitive to rheumatoid factor disturbances.
No assay interference was demonstrated with DIMERTEST® Latex with spiked specimens containing potential interferents at the following concentrations:

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