

D-DIMER LATEX AGGLUTINATION KIT

Diagnostic Reagent for the rapid qualitative or semi-quantitative evaluation of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human plasma

REF	Cont.	
T9515707	1 x 2,7 ml	D-Dimer Latex
	1 x 25 ml	D-Dimer Buffer
	1 x 0,5 ml	Positive Control
	1 x 0,5 ml	Negative Control
	5 pcs.	Testcards
	50 pcs.	Mixing sticks

GENERAL INFORMATION

Method: Latex Agglutination
 Temperature: 37°C
 Sample: Sodium Citrate Plasma

Number of Tests

80 Tests manual method

REAGENT COMPOSITION

D-dimer latex:

Suspension of latex beads which are coated with murine anti-D-Dimer monoclonal antibody, 10 mg/ml BSA and 0.1% sodium azide .

D-Dimer Buffer:

10 mM phosphate Buffer solution with 0.1% sodium azide

Positive Control:

Solution containing purified human D-Dimer fragment, 5 mg/ml BSA and 0.1% sodium azide.

Negative Control:

Buffer solution containing 5 mg/ml BSA and 0.1% sodium azide.

REAGENT PREPARATION

D-dimer latex:

Ready to use. Mix by inversion immediately before use!

D-Dimer Buffer:

Ready to use.

Positive Control:

Ready to use.

Negative Control:

Ready to use.

REAGENT STABILITY AND STORAGE

Conditions: protect from light
 close immediately after use
 Storage: at 2 – 8°C
 Do not freeze!

Reagent deterioration is indicated by failure of Latex Reagent to agglutinate with the positive control, agglutination with negative control, or evidence of microbiological contamination.

SAMPLE COLLECTION AND PREPARATION

Plasma 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 (1500g for 15 minutes at 4-10°C), specimens may be tested directly for the presence of XL-FDP. Defibrination of plasma is recommended.

SAMPLE STABILITY AND STORAGE

Stability: 2 weeks at -20°C
 Thaw frozen specimens rapidly at 37°C and centrifuge before testing.

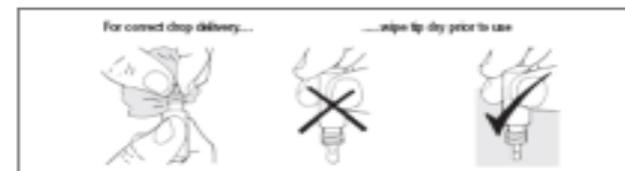
INTERFERING SUBSTANCES

No interference was demonstrated with Dialab D-Dimertest Latex with spiked specimens containing potential interferences at the following concentrations:

Bilirubin	0.2 mg/ml
Triglycerides	30 mg/ml
Hemoglobin	5.0 mg/ml
Protein	0.06 mg/l

MANUAL TEST PROCEDURE

Allow all vials to warm to room temperature before use. Prior to use, the dropper bottle tips must be wiped dry with a tissue. Dropper bottles must be held vertically when dispensing drops of reagent.



Qualitative Method:

Place on a test card	Sample	Pos. Ctrl.	Neg. Ctrl
Sample	20 µl	-	-
Pos. Ctrl.	-	1 drop	-
Neg. Ctrl	-	-	1 drop

Place in a nearby area of each circle:

D-Dimer Latex	1 drop	1 drop	1 drop
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Mix the Latex reagent and sample (control) with a stirrer until the Latex is uniformly distributed. Rock the test card gently by hand for exactly 3 minutes.

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 false agglutination pattern. If this is suspected, the specimen must be retested.

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.

Semi-quantitative method

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

Approximate levels of XL-FDP, containing the D-Dimer domain, for specimens dilution are shown Table 1. As with all semi-quantitative 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

EXPECTED VALUES

A positive result, indicating active fibrinolysis, should be obtained with DIALAB D-Dimer Latex when XL-FDP (D-Dimer) levels 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 to many variables that may effect results, each laboratory should establish its own normal range.

Elevated levels of XL-FDP (containing the D-Dimer domain) have been demonstrated in patients by a combination of immunoprecipitation and gel electrophoresis technique. Monoclonal antibodies allow the specific detection of the D-Dimer domain. Monoclonal antibody based D-Dimer assays are of diagnostic value in disseminated intravascular coagulation (DIC) and acute vascular disease, including pulmonary embolism (PE) and deep venous thrombosis (DVT), conditions that are difficult to detect reliably by clinical examination.

The amount of XL-FDP detected in a specimen will depend on several interrelated factors in vivo, 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 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. D-Dimer levels also rise during normal pregnancy but very high levels are associated with complications. DIALAB D-Dimer Latex does not cross-react with fibrinogen, factor XIIIa cross-linked fibrinogen, or fibrinogen degradation products.

TEST PRINCIPLE

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

DIAGNOSTIC IMPLICATIONS

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. Therefore, cross-linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

Clinical diagnosis should not be based on the result of DIALAB D-Dimer Latex alone. Clinical signs and other relevant test information should be induced in the diagnostic decision.

PERFORMANCE CHARACTERISTICS SPECIFICITY

Plasma from 170 apparently healthy, voluntary blood donors was tested using DIALAB D-Dimer Latex. A negative result was obtained for 162 of the samples. This equates to a specificity of 95.3% (162/17).

PRECISION

Intra –Assay Precision

Intra-assay reproducibility was determined for 10 replicates of 3 plasma samples that contained different levels of XL-FDP. The results were equivalent for all replicates.

Inter –Assay Precision

Inter-assay reproducibility was determined using 10 plasma samples with XL-FDP titers ranging from 1 to 16. In 10 runs, the replicates of these specimens did not vary by more than one titer.

METHOD COMPARISON

145 plasma samples from patients judged to be suffering from, or having a high probability for thrombotic episode, were tested by DIALAB D-Dimer Latex and another agglutination reference method. The correlation coefficient was $r = 0.94$ and the regression equation was $y = 1.19x$.

In an anticoagulant study of 50 parallel citrated, EDTA and heparin plasma samples, the correlation between the titers obtained with DIALAB D-Dimer 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.

QUALITY CONTROL

The positive and negative controls provided in the kit should be used for quality control of the assay. Controls should be tested in the same way as patient samples.

WARNINGS AND PRECAUTIONS

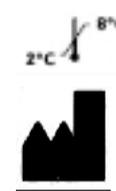
- For in vitro diagnostic use only
- Harmful if swallowed, avoid contact with skin and eyes, do not empty into drains, wear suitable protective clothing
- Caution: all reagents in DIALAB D-Dimer test latex contain sodium azide (0.1%) as preservative. DO not ingest or allow to contact skin or mucous membranes. Sodium azide may form explosive azides in metal plumbing. Use proper disposal procedures.
- Each unit of source plasma used in the preparation of this product has been tested by an FDA approved method for the presence of antibody to Human Immunodeficiency Virus (HIV) Type I and Type II, Hepatitis B surface antigen (HBsAg) as well as for Hepatitis C (HCV) and found negative (not repeatedly reactive). However, no test can offer complete assurance that products derived from human blood will not transmit infectious diseases. As with all materials of human origin, this product should be handled as a potentially infectious material.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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