INSTRUCTION MANUAL

REF **3021**

April 01, 2014

D-Dimer Latex

- 50 determinations -

CE

IVD *In vitro* diagnostic device

Latex agglutination test for the detection of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human plasma

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

D-Dimer Latex is used for the rapid qualitative or semi-quantitative detection of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human plasma..

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.

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2. Lane, D.A. et al. Characterisation of Serum Fibrinogen and Fibrin Fragments Produced During Disseminated Intravascular Coagulation. Br. J. Haematol. 40 (4): 609-615; 1978.
3. Whitaker, A.N. et al. Identification of D-Dimer-E complex in Disseminated Intravascular Coagulation. Thromb. Res. 18 (3-4): 453-459; 1980.
4. NCCLS Publication H21-A3 - Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays; Approved Guideline Third Edition; 1998.
5. Graeff, H. et al. Detection and Relevance of Crosslinked Fibrin Derivatives in Blood. Semin. Thromb. Hemost. 8 (1): 57-68; 1982.
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7. Rylatt, D.B. et al. An Immunoassay for Human D-Dimer using Monoclonal Antibodies. Thromb. Res. 31 (6): 767-778; 1983.
8. Elms, M.J. et al. Rapid Detection of Cross-Linked Fibrin Degradation Products in Plasma using Monoclonal Antibody-Coated Latex Particles. Am. J. Clin. Pathol. 85 (3): 360-364; 1986.
9. Whitaker, A.N. et al. Measurement of Cross-Linked Fibrin Derivatives in Plasma: an Immunoassay using Monoclonal Antibodies. J. Clin. Pathol. 37 (8): 882-887; 1984.
10. Hunt, F.A. et al. Serum Crosslinked Fibrin (XDP) and Fibrinogen/Fibrin Degradation Products (FDP) in Disorders Associated with Activation of the Coagulation or Fibrinolytic Systems. Br. J. Haematol. 60 (4): 715-722; 1985.
11. Smith, R.T. et al. Fibrin Degradation Products in the Postoperative Period. Evaluation of a New Latex Agglutination Method. Am. J. Clin. Pathol. 60 (5): 644-647; 1973.
12. Nolan, T.E. et al. Maternal Plasma D-Dimer Levels in Normal and Complicated Pregnancies. Obstet. Gynecol. 81 (2): 235-238, 1993.

PRINCIPLE OF THE TEST

D-Dimer Latex is used for the qualitative and semi-quantitative determination of D-Dimer in human plasma.

D-Dimer 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 occurring when the concentration of D-Dimer is above the threshold of detection of the assay.

TEST COMPONENTS for 50 determinations

A **LATEX**	**Latex reagent** Latex particles coated with monoclonal anti-D-Dimer antibodies (monoclonal, murine)		1.0 ml ready for use
B **BUF**	**Sample diluent**		10 ml ready for use
P **CONTROL**	**Positive control** D-Dimer positive human plasma	**+**	0.5 ml ready for use
N **CONTROL**	**Negative control** Negative human plasma	**-**	0.5 ml ready for use
	Agglutination slide		1 ready for use
	Disposable stirring sticks		50 ready 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)

Size and storage

D-Dimer Latex has been designed for 50 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the D-Dimer Latex have to be stored at
2 - 8 °C, preferably in the original kit box. The D-Dimer Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal. **Do not freeze!**

Do not use the latex reagents if it is marked with turbidity as this may indicate reagent deterioration or contamination.

After opening all kit components are stable for at least 3 months, provided proper storage.

Agglutination slide should be thoroughly rinsed with water and wiped with lint-free tissue after each use.

PATIENT SAMPLES

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 (1500g for 15 minutes at 4°C - 10°C), specimens may be tested directly for the presence of XL-FDP. Defibrination of the plasma is not recommended.

Plasma samples are stable when stored at -20ºC for up to: 2 weeks. Thaw frozen specimens rapidly at 37ºC and centrifuge before testing.

ASSAY PROCEDURE

Qualitative evaluation

1. Allow all reagents and samples to reach room temperature prior to testing. Shake well all reagents before use.
2. pipette **20 µl** of the latex reagent (A) within a well on a Agglutination slide. AVOID touching the surface of the Agglutination slide.
3. Accurately pipette **20 µl** of undiluted plasma or of control solution inside the same well next to the drop of Latex Reagent.
4. Mix the Latex Reagent (A) and sample with a stirrer until the Latex is uniformly distributed.
5. Rock the Agglutination slide 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.**

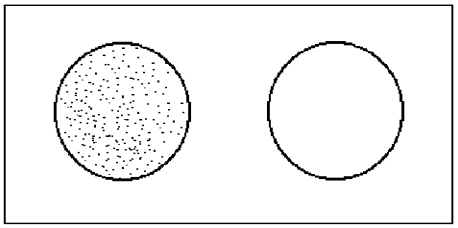
Semi-quantitative evaluation

1. Allow all reagents and samples to reach room temperature prior to testing. Shake well all reagents before use.
2. Prepare serial dilutions of the test plasma with Dilution Buffer (B) as follows:
   1:2 dilution 100 µL plasma plus 100 µL Buffer solution (B)
   1:4 dilution 100 µL 1:2 dilution plus 100 µL Buffer solution (B)
   1:8 dilution 100 µL 1:4 dilution plus 100 µL Buffer solution (B)
3. Test each dilution as described in the qualitative method.

EVALUATION OF RESULTS

POSITIVE
A positive reaction is indicated by any observable agglutination in the reaction mixture. A weakly reactive serum produces a very fine granulation or a partial clumping. The specimen reaction should be compared to the Positive control.

NEGATIVE
A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the Negative control.



Positive Negative

Qualitative test evaluation

For the qualitative assay protocol, the following results should be obtained:
D-Dimer (XL-FDP) concentration in undiluted Plasma:
Negative: less than 0.20 mg/l (200ng/ml)
Positive: greater than 0.20 mg/l (200ng/ml)

Note: All values in mg/l (ng/ml) are approximate values.

Semi-quantitative test evaluation

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.

Approximate Range of D-Dimer (XL-FDP) mg/l (ng/ml)	Sample Dilution			
	Undil.	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.

Test validity

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.
D-Dimer Positive Control consists of a solution of human D-Dimer at a level of approximately 0.80 mg/L (800ng/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). 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 (200ng/mL). Due to many variables that may affect 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 techniques. Monoclonal antibodies allow the specific detection of the D-Dimer domain. Monoclonal antibody based D-Dimer assay is 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 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 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. D-Dimer levels also rise during normal pregnancy but very high levels are associated with complications.

D-Dimer Latex does not cross-react with fibrinogen, factor XIIIa cross-linked fibrinogen, or fibrinogen degradation products.

Limitations of the method

Reaction time is critical. If reaction time exceeds 3 minutes, drying of the reaction mixture may cause false positive result.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance characteristics

Plasma from one hundred and seventy (170) apparently healthy, voluntary blood donors was tested using D-Dimer Latex. A negative result was obtained for one hundred and sixty-two (162) of the samples. This equates to a specificity of 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 D-Dimer Latex and another agglutination reference method. The correlation 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 levels 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 16. 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 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.

No assay interference was demonstrated with D-Dimer Latex with spiked specimens containing potential interfering substances 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

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed.
- Do not use or mix reagents from different lots. Do not use reagents from other manufacturers.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Sodium azide (0.095%) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Positive and negative controls prepared using human sera found negative for hepatitis B surface antigen (HBsAg) and antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) by FDA required test. However, handle controls as if potentially infectious.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.