remel

Apogent

D-dimer E

INTENDED USE

D-dimer is a rapid assay for the qualitative and semi-quantitative measurement of cross-linked fibrin degradation products (XDP) in serum or plasma. D-dimer has been categorized as moderately complex under the Clinical Laboratory Improvement Act (CLIA 88).

SUMMARY AND EXPLANATION OF THE TEST

Thrombi, present in thromboembolic disease states, consist of cross-linked fibrin. The first stage in clot formation is the conversion of fibrinogen to fibrin monomer by the action of thrombin. Thrombin also activates Factor XIII to Factor XIIIa which, in turn, catalyses the formation of covalent bonds between the "gamma" chains of fibrin monomers, thus forming the D-dimer (DD) derivative. Cross-linking also occurs between the α chains of the fibrin monomers by a slower process[1,6].

Because of the formation of these covalent bonds, the cross-linked fibrin, on digestion with plasmin, gives rise to several degradation products (XDP) which are molecularly distinct from those of fibrinogen[3,4]. The terminal plasminolytic cleavage product of cross-linked fibrin is the D-dimer fragment, which is often found to be associated, by non-covalent bonds, with fragment E, making a (DD)E complex[1]. The presence of molecules containing the DD epitope in serum or plasma is therefore a marker for secondary fibrinolysis.

D-dimer is a slide agglutination test in which one drop of sample and one drop of latex suspension are mixed for three minutes by gentle rocking. An agglutinated pattern at the end of the test period indicates the presence of XDP in the sample under test. Since the sensitivity of the test is set at 1 µg (DD)E per ml, by testing samples at several different dilutions an approximate XDP level can be determined.

PRINCIPLE OF THE TEST

A monoclonal antibody[9] raised against a highly purified preparation of human DD fragments has been used to coat a suspension of latex particles. The coated latex particles agglutinate with cross-linked fibrin degradation products, (DD)E, DY/YD, YY/DXD, YXD/DXY but not with fibrinogen or fibrinogen degradation products, X, Y, D or E.

The sensitivity of the latex reagent is adjusted, during manufacture, to 1 µg per ml using a purified (DD)E preparation as a standard.

REAGENTS

KIT CONTENTS

D-Dimer	50 tests
1. Latex Suspension	1 bottle
2. Diluent	1 bottle
3. Positive Control	2 bottles
4. Disposable Reaction Cards	12
5. Disposable Mixing Sticks	60
6. Disposable Droppers	60
7. Instructions for Use	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.

The reagents should be stored at 2 to 8°C, both before and after reconstitution. The Latex Suspension, Diluent and unreconstituted Positive Control will retain their activity until the expiry date of the kit, when stored in this way. Once reconstituted the Positive Control will remain stable for up to 12 weeks stored at 2 to 8°C or for 3 months when stored at –20°C or colder.

LATEX

Latex Suspension

1 dropper bottle containing 2.1 ml of a 1.0% suspension of polystyrene latex particles coated with anti-DD mouse monoclonal antibody containing 0.1% of Topcide 300* preservative.

DILUENT

Diluent

1 bottle containing 9.5 ml of Tris-Saline buffer containing 0.1% of Topcide 300* preservative.

CONTROL + ⚠

Positive Control

2 bottles each containing the freeze-dried equivalent of 0.5 ml of a solution of purified human (DD)E complex in a human serum base. The reagent contains 1.57% sodium azide before reconstitution and 0.1% when reconstituted.

Tap the bottle gently on the bench to remove any material adhering to the rubber stopper. Carefully remove the stopper and add 0.5 ml of distilled or deionised water to the bottle of freeze dried Positive Control. Replace the stopper and allow to stand for a few minutes with occasional swirling and inversion to aid dissolution.

WARNINGS AND PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

HEALTH AND SAFETY INFORMATION

1. Human Serum based materials used in the manufacture of this kit were from single donations which have been tested for HBsAg and antibodies to HIV-1, HIV-2 and HCV and found to be negative. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.

2. The Positive Control contains 1.57% sodium azide, before reconstitution, which is classified per applicable European Economic Community (EEC) Directives as very toxic (T+). The following are the appropriate Risk (R) and Safety (S) phrases.

T+	**R26/27/28**	Very toxic by inhalation, in contact with skin and if swallowed
	**R32**	Contact with acid liberates toxic gas
	R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
	S20	When using do not eat or drink
	S28	After contact with skin, wash immediately with plenty of water
	S29/35	Do not empty into drains; dispose of this material and its container in a safe way
	S36/37/39	Wear suitable protective clothing, gloves and eye protection
	S43b	In case of fire, use water or powdered extinguishing agent
	S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.

3. In accordance with the principles of Good Laboratory Practice it is strongly recommended that all human based materials, whether they be samples from patients or Positive Control should be handled as potentially infectious and used with all necessary precautions.
4. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 1 hour at 121°C; disposables should be autoclaved or incinerated. Spillage of potentially infective materials or of control samples should be removed immediately with absorbent paper tissues and the contaminated areas swabbed with a standard disinfectant, 70% alcohol or a solution of 0.5% (minimum concentration) sodium hypochlorite. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste. Do not autoclave waste containing sodium hypochlorite.
5. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
6. When used in accordance with the principles of Good Laboratory Practice, Good Standards of Occupational Hygiene and the instructions stated in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS

1. Do not use reagents beyond the stated expiry date. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
2. NOTE: Allow all reagents to reach room temperature (18 to 30°C) before use.
3. Do not allow the Latex Suspension or Diluent to freeze.
4. Care should be taken to fully re-suspend the latex before use.
5. Avoid contamination of the reaction circles of the Disposable Reaction Cards, e.g. by fingerprints or scratching the surface with the mixing sticks. This could result in unreliable test results.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

SPECIMEN COLLECTION

1. The D-dimer kit may be used to test serum or plasma samples.
2. Care should be taken to ensure that the samples are free from haemolysis and/or contamination.
3. Whilst care should be taken to ensure that serum samples are fully clotted, the serum must be separated from the clot as soon as possible. If necessary, the serum should be clarified by centrifugation before testing.
4. Plasma samples may be collected in citrate or EDTA and, if necessary, should be clarified by centrifugation before testing. The plasma should be separated from the cells as soon as possible after collection.

SPECIMEN TRANSPORT AND STORAGE

1. Separated samples may be stored for up to 7 days at 2 to 8°C. If longer storage is required the samples should be stored at –20°C or colder where they will remain stable for up to 2 months. Samples may be frozen and thawed twice with no detrimental effect.

PROCEDURE

MATERIALS PROVIDED

The D-dimer kit contains sufficient reagents to perform 50 tests, see **Kit Contents**.

EQUIPMENT REQUIRED BUT NOT PROVIDED

1. A timer or stopwatch.
2. A micropipette to deliver 0.5 ml.
3. Distilled or deionised water.
4. A mechanical rotator of the oscillating type (optional). The rotator should mix at 50 to 60 rpm and have a table that inclines to 20 degrees.
5. For semi-quantitative test only:
   a) Tubes in which to perform dilutions.
   b) A micropipette to deliver 0.1 ml.

TEST PROCEDURE

a) **Qualitative Procedure**

Step 1 Using the dropper provided add one drop (35 µl) of sample or control to a circle on the Reaction Card.

Step 2 Resuspend the Latex by gently swirling and inverting the bottle several times then add one drop (35 µl) of the suspension to the reaction circle, taking care not to contaminate the nozzle of the dropper with sample.

Step 3 Using the mixing sticks provided stir and spread the latex/sample mixture over the entire area of the circle.

Step 4 Either manually or using a mechanical rotator, rotate the Reaction Card for exactly 3 minutes and examine for macroscopic agglutination.

Step 5 Read the results immediately after rocking the card for 3 minutes. If the reaction is allowed to continue for longer, false results may occur due to drying of the mixture on the card.

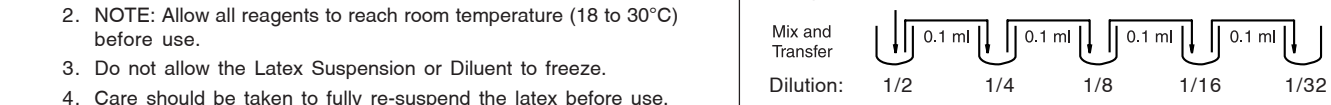
See **Reading the Results** section.

b) **Semi-quantitative Procedure**

Step 1 When the undiluted sample is positive, an estimation of the level of XDP in the sample can be obtained by preparing a doubling dilution series from 1/2 to 1/32 as follows:

Step 2

Tube:	No. 1	No. 2	No. 3	No. 4	No. 5
Diluent:	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Sample:	0.1 ml				



Dilution:	1/2	1/4	1/8	1/16	1/32

Step 3 Using the droppers provided, transfer 1 drop of each dilution starting from 1/32 to separate positions on the reaction card.

Step 4 Resuspend the Latex by gently swirling and inverting the bottle several times and then add one drop (35 µl) of the suspension to each reaction circle, taking care not to contaminate the nozzle of the dropper with sample.

Step 5 Using the mixing sticks provided stir and spread each of the Latex/sample mixtures in turn over the entire area of the circle.

Step 6 Either manually or using a mechanical rotator, rotate the Reaction Card for exactly 3 minutes and examine for macroscopic agglutination.

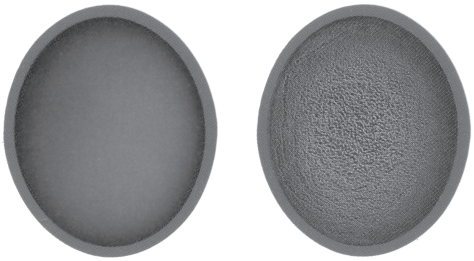
Step 7 Read the results immediately after rocking the card for 3 minutes. If the reaction is allowed to continue for longer, false results may occur due to drying of the mixture on the card. See **Reading the Results** and **Calculation of Results – Semi-quantitative Procedure** sections.

RESULTS

READING THE RESULTS

As with all slide agglutination reactions, the results are best viewed in bright diffuse daylight, but the patterns obtained with D-dimer can easily be recognised under any normal lighting conditions. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens.

Figure 1 Figure 2



A POSITIVE reaction is indicated when visible clumps of latex are formed within 3 minutes of mixing the latex suspension with the sample (i.e. an agglutinated pattern – see Figure 2). However, the actual quality of agglutination observed will depend on the concentration of antigen in the sample and will vary from large clumps which develop within a few seconds of mixing, to small clumps which develop rather slowly.

In a NEGATIVE reaction the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the 3 minute test (see Figure 1). Note, however, that faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator.

CALCULATION OF RESULTS – SEMI-QUANTITATIVE PROCEDURE

XDP levels can be approximated from the results obtained by referring to Table 1. Note that the results are expressed in terms of (DD)E.

Table 1

Results Obtained*

Sample Dilution						Approximate XDP Level in µg/ml
Neat	1/2	1/4	1/8	1/16	1/32	(DD)E
+	–	–	–	–	–	1-2
+	+	–	–	–	–	2-4
+	+	+	–	–	–	4-8
+	+	+	+	–	–	8-16
+	+	+	+	+	–	16-32
+	+	+	+	+	+	>32

* + = agglutinated pattern – = negative pattern. Further dilutions may be performed if necessary.

QUALITY CONTROL

The Positive Control is provided to ensure the proper functioning of the test. Either the Diluent or a previously assayed negative specimen should be used as a negative control. Positive and negative control samples should be run at least once each day the test is used. The Positive Control should give a clearly positive reaction (see **Reading the Results**) and when the diluent (or negative specimen) is tested the result should be negative.

If the above two criteria are not met then any results are invalid. The procedure should then be examined to ensure that the assay was performed correctly and the test repeated. If the test still fails to meet the criteria the reagents must not be used.

INTERPRETATION OF RESULTS

The normal circulating level of XDP in healthy adult subjects is usually less than 1 µg (DD)E/ml as measured by this test (see **Specific Performance Characteristics**). Thus, samples from subjects with normal circulating levels are expected to give a negative result in the test.

Elevated levels of XDP have been demonstrated, using several different assay techniques, in samples from patients with various disease states. These include Disseminated Intravascular Coagulation (DIC)[5,6,7], Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE)[2].

LIMITATIONS OF THE PROCEDURE

1. The assay range of the kit is 1-256 µg of (DD)E per ml.
2. The results obtained using the semi-quantitative procedure are approximate values.
3. A diagnosis should not be made based on an XDP result alone. The clinical symptoms shown by the patient must be taken into account before a diagnosis is made.
4. Contaminated or haemolysed serum/plasma samples are not suitable for testing.
5. Samples which have been stored unseparated are unsuitable for testing.
6. Separated samples showing signs of clotting are not suitable for testing.
7. It has been demonstrated[8] that the levels of XDP measured in serum are often lower than those in the equivalent plasma sample.
8. Eighteen sera with Rheumatoid Factor levels from 9.5 Units/ml to 445 Units/ml were tested with D-dimer. All 18 samples gave a negative reaction.

EXPECTED RESULTS

A positive result will be seen in samples containing more than or equal to 1 µg/ml XDP.

SPECIFIC PERFORMANCE CHARACTERISTICS

Parallel serum, EDTA and citrated plasma samples collected from 49 healthy volunteers were tested with D-dimer. A negative result was obtained for 47 of each type of sample. This equates to a specificity of 95.9% (47/49) for the three types of samples collected. One hundred and five citrated plasma samples known to be positive for cross-linked fibrin degradation products with another commercially available test, were tested with D-dimer. Clinical conditions associated with risk for disseminated intravascular coagulation disorder were identified for 96 of the 105 patients involved. These included (numbers in brackets), surgery/trauma (29), cardiovascular/pulmonary disease (19, including 1 pulmonary embolism), hepatic disease (10), obstetric complications (5), renal disease (4), gastrointestinal disease (12), neoplasm (6), leukaemia (5), sepsis (2), neurological disease (2) and viral illness (2). One hundred and three of these samples were positive with D-dimer. The two samples (one gastrointestinal disease, and one cardiovascular disease) which D-dimer found to be negative were also negative in a second commercially available test. Twenty citrated plasma samples from snake bite victims were tested semi-quantitatively with D-dimer in parallel with another commercially available test. The XDP concentration (1 – >32 µg/ml) obtained with D-dimer was equivalent to the XDP concentration (0.5 – >32.0 µg/ml) obtained with the other test for each sample tested. Forty-eight paired serum and citrated plasma samples collected from patients were tested semi-quantitatively with D-dimer. Forty of the samples gave the same titre whether serum or plasma was used, 2 samples gave a higher result by one dilution when serum was used and 6 samples gave a higher result by one dilution when plasma was used. Two of the latter 6 samples were weakly positive when plasma was used and negative with serum. (See **Limitations of the Procedure**).

Assay Reproducibility

An XDP-containing sample was titrated in 12 separate assay runs (3 operators). The titres obtained were all within one doubling dilution. A further sample was titrated on 5 occasions with one batch of reagents and 5 occasions with a second batch. The titre obtained was the same in each of the 10 assays. A negative sample and a positive sample were tested, using the qualitative test procedure, 5 times with each of 3 batches of reagents. The expected results were obtained on each occasion.

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PACKAGING

REF HA10/30852501.....50 tests

Symbol legend

REF	Catalog Number
IVD	*In vitro* diagnostic medical device
	Consult instruction for use (IFU)
	Temperature limitation (Storage Temp.)
LOT	Batch code (Lot Number)
	Use by (Expiration Date)
	Caution, consult accompanying documents

*trade mark.

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