

Catalogue # 1431 (ROW)

Auto•Dimer®

Quantitative, automated Latex
Agglutination Test for Fibrin D-dimer.

For *in vitro* diagnostic use
Lot # L13940

I. INTENDED USE

Auto•Dimer® is an immunoturbidimetric assay used for the quantitative determination of the fibrin degradation products that contain D-dimer in human plasma on the instruments Hitachi 911, Hitachi 902, and Thrombolyzer Compact X/Compact XR/Combi/RackRotor™.

II. SUMMARY

D-dimer containing moieties are formed by plasmin degradation of factor XIIIa cross-linked fibrin. Elevated levels of D-dimer are found in clinical conditions such as deep vein thrombosis (DVT), pulmonary embolism (PE) and disseminated intravascular coagulation (DIC).¹⁻³ Laboratory measurements of fibrin degradation products, including D-dimer, have significance in the initial assessment of these conditions.

III. PRINCIPLE

Biopool Auto•Dimer® is a turbidimetric assay that utilizes antibody coated latex particles. In the presence of D-dimer, the particles aggregate and turbidity increases. The increase in scattered light is proportional to the amount of D-dimer in the sample. The latex particles are coated with a monoclonal antibody that reacts with fibrin D-dimer or fragment D of fibrin. The antibody has no cross reactivity with fibrinogen.⁴ This allows for the determination of D-dimer in human plasma.

IV. REAGENTS

A. Reagent description

The reagents are lot-specific. Lots are not interchangeable.

1. Latex Reagent

1 x 13.5 ml latex particles coated with anti-D-dimer monoclonal antibody MA-8D3 suspended in HEPES buffer pH 8.5, containing stabilizers, detergent and sodium azide.

2. Reaction Buffer

1 x 22 ml HEPES buffer, pH 7.0, containing stabilizers, detergent and sodium azide.

3. Saline Solution

2 x 8 ml buffered saline, pH 7.3, containing sodium azide.

4. D-dimer Standard 0 µg/L

2 x 1 ml lyophilized human plasma immunodepleted of D-dimer.

5. D-dimer Standard 3200 µg/L

2 x 1 ml lyophilized human plasma enriched with D-dimer.

Lot-specific assayed concentration:

Hitachi 911/902: 3228 µg/L.

Thrombolyzer: 3.23 mg/L.

B. Reagent Preparation

1. Latex Reagent

Ready to use. The latex may sediment during storage. Mix thoroughly before use.

2. Reaction Buffer

Ready to use.

3. Saline solution

Ready to use.

4. D-dimer standard 0 µg/L

Hitachi 911/902 application: Reconstitute with 1.0 ml of Saline Solution and agitate gently for 5 minutes to completely dissolve contents.

Thrombolyzer application: The reagent is not used.

5. D-dimer standard 3200 µg/L

Hitachi 911/902 application: Reconstitute with 1.0 ml of Saline Solution and agitate gently for 5 minutes to completely dissolve contents.

Thrombolyzer application: Reconstitute with 1.4 ml of Saline Solution and agitate gently for 5 minutes to completely dissolve contents. **Note:**

This procedure will give the D-dimer concentration of approximately 2.40 mg/L.

V. STORAGE AND STABILITY

The unopened and unreconstituted reagents are stable until the expiration date stated on the box and vial labels when stored at 2-8 °C.

1. Latex Reagent

Store at 2-8 °C and use within 4 weeks from opening.

Instrument on-board storage:

Hitachi 911/902 application: Stable for 4 weeks when stored on-board.

Thrombolyzer application: Stable for 1 week when stored on-board.

2. Reaction Buffer

Store at 2-8 °C and use within 4 weeks from opening.

Instrument on-board storage:

Hitachi 911/902 application: Stable for 4 weeks when stored on-board.

Thrombolyzer application: Stable for 1 week when stored on-board.

3. Saline solution

Store at 2-8 °C and use within 4 weeks from opening.

4. D-dimer standard 0 µg/L

Stable for 10 hours at 20-25 °C.

5. D-dimer standard 3200 µg/L

Stable for 10 hours at 20-25 °C.

VI. WARNINGS AND PRECAUTIONS

The Biopool Auto•Dimer® Standards are of human origin. Each donor unit of source plasma used in these products has been tested and found negative for Hepatitis B antigens, HIV I and II antibodies, Hepatitis C antibodies, syphilis antibodies and H.T.L.V. I/II antibodies by FDA approved methods. However no test can offer complete assurance that products derived from human blood will not transmit infectious disease. As with all materials of human origin, this product should be handled as a potentially infectious agent. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

The Latex Reagent, Reaction Buffer and Saline Solution contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Materials discarded into a sink should be flushed with a large volume of water to prevent azide build-up.

The test should be used in conjunction with clinical observations and results of other laboratory tests.

VII. SPECIMEN COLLECTION

Collect samples in nine volumes of blood and one volume of 3.2% buffered sodium citrate (0.105 M). Centrifuge at 3000 x g for 10 min. Citrated plasma samples may be stored at room temperature for 2 hours or at 2-8 °C for 18 hours. A single freeze-thaw cycle does not affect the assay response. Refer to NCCLS H21-A2 for specific sample collection guidelines.

VIII. PROCEDURE

A. Material Provided

Latex Reagent

Reaction Buffer

Saline Solution

D-dimer Standards, 0 and 3200 µg/L

Instrument applications for Hitachi 911/902 and Thrombolyzer

B. Materials required but not provided

Automatic analyser (Hitachi 911, Hitachi 902, or Thrombolyzer Compact X/Compact XR/Combi/RackRotor™)

D-dimer Low Control, Biopool catalogue # 1436

D-dimer High Control, Biopool catalogue # 1468

Pipettes for 125 µl and 1000 µl

Pipette tips

Test tubes

1. Instrument applications

The reagents of the Auto•Dimer® kit are intended to be used on the Hitachi 911, Hitachi 902, or Thrombolyzer Compact X/Compact XR/Combi/RackRotor™ instruments. For detailed instructions, see instrument-specific application sheets.

2. Preparation of Standard Curve

Prepare the standard dilutions as described below.

The standard dilutions are stable for 10 hours at 20-25 °C.

Use the lot-specific value for D-dimer Standard 3200 µg/L given in the Reagent Description section to determine the exact D-dimer concentration in each standard dilution.

Users must construct a standard curve each time a new kit lot is used, or every 6 months, and if Control Plasma is assayed out of range. Typical standard curves are shown in the instrument-specific application sheets.
Hitachi 911/902 application: Make a serial dilution of the D-dimer Standard 3200 µg/L with the D-dimer Standard 0 µg/L according to the table. Note that Standard Dilution E is not used for the construction of the standard curve.

Standard Dilution	Lot-specific conc. (µg/L)	0 µg/L standard	Mix with
A	3228	0	---
B	1614	125	125 µl Std Dilution A
C	807	125	125 µl Std Dilution B
D	404	125	125 µl Std Dilution C
E	202	125	125 µl Std Dilution D
F	101	125	125 µl Std Dilution E
G	0	125	---

Thrombolyzer application: The dilutions are made by the instrument using Saline Solution as diluent. The following dilutions should be programmed:

Programmed dilution	Lot-specific conc. (mg/L)
1:1	3.23
1:2	1.61
1:4	0.81
1:8	0.40
1:16	0.20

3. Assay procedure

See instrument-specific application sheets.

IX. RESULTS

Hitachi 911/902 application: The results are reported in µg/L D-dimer.

Thrombolyzer application: The results are reported in mg/L D-dimer. Conversion of results to µg/L: 1 mg/L = 1000 µg/L.

X. QUALITY CONTROL

It is recommended that the Control Plasmas D-dimer Low Control and D-dimer High Control are assayed at regular intervals in order to ensure consistent assay results. If the control plasma result deviates from the D-dimer concentration given in the lot-specific Instruction for Use, a new standard curve should be constructed.

XI. LIMITATIONS AND INTERFERENCES

Presence of rheumatoid arthritis factor may result in false-positive results (influence not quantified).

Hitachi 911/902 application: No interference is found from bilirubin (<0.27 g/L), hemoglobin (<6.7 g/L), or triglycerides (17 g/L). Highly lipemic samples should be diluted in Saline Solution and re-assayed.

Thrombolyzer application: No interference is found from bilirubin (<0.27 g/L), hemoglobin (<6.7 g/L), or triglycerides (3.3 g/L). Highly lipemic samples should be diluted in Saline Solution and re-assayed.

XII. EXPECTED VALUES

Hitachi 911/902 application: In a study of 80 (Hitachi 911) and 30 (Hitachi 902) normal individuals, 95% of the values were below 110 µg/L.

Thrombolyzer application: In a study of 30 normal individuals, 95% of the values were below 0.15 mg/L (150 µg/L).

Elevated levels are found in patients with confirmed deep venous thrombosis (DVT), pulmonary embolism, DIC, and trauma.¹⁻³ D-dimer levels rise during pregnancy and high levels are associated with complications.⁵

The concentration of D-dimer in any given specimen may differ from the concentration determined using D-dimer assays from different manufacturers. Consequently, each laboratory must determine reference intervals for their individual test populations, reagents, and instruments.

XIII. PERFORMANCE CHARACTERISTICS

The user should establish product performance characteristics for the specific instrumentation used.

All D-dimer values below are expressed in µg/L.

A. Correlation

When compared to another quantitative immunoturbidimetric method (Biopool MiniQuant[®] D-dimer) the Auto•Dimer[®] assay (Hitachi 911) correlated as follows:

$$y \text{ (Auto•Dimer[®] Hitachi 911)} = 0.92 \times \text{(MiniQuant[®] D-dimer)} - 13.0, \\ r^2 = 0.943, n = 213 \text{ samples}$$

The different Auto•Dimer[®] applications correlated as follows:

$$y \text{ (Auto•Dimer[®] Hitachi 902)} = 1.05 \times \text{(Auto•Dimer[®] Hitachi 911)} - 50.4, \\ r^2 = 0.999, n = 30 \text{ samples}$$

$$y \text{ (Auto•Dimer[®] Thrombolyzer)} = 1.07 \times \text{(Auto•Dimer[®] Hitachi 911)} - 43.8, \\ r^2 = 0.988, n = 30 \text{ samples}$$

B. Precision

The precision was determined by assaying D-dimer control plasmas at two different D-dimer levels in 5 replicates at 7 occasions.

Within run precision (n=35)	Hitachi 911 CV (%)	Hitachi 902 CV (%)	Thrombolyzer CV (%)
Low	1.2	2.5	3.3
High	0.5	0.5	6.4
Between run precision (N=7, n=5)			
Low	6.0	1.8	2.5
High	3.1	0.7	0.9

C. Lot-to-lot reproducibility

Five in-house reference plasmas containing D-dimer levels distributed over the whole assay range were analyzed with 5 different reagent lots. All between lot CV's were ≤ 5%.

D. Accuracy/Recovery

Serial dilutions of five patient plasmas resulted in linear dose-response curves with correlation coefficients (r^2) greater than 0.996 for all instruments. The recovery, expressed as the slope of the regression between expected and recovered values, was 1.00 ± 0.15 in all cases.

E. Sensitivity and Assay Range

Hitachi 911/902 application: The lower limit of detection (defined as mean of the blank + 3SD) is 80 µg/L. The assay range is 100-3200 µg/L. Samples above 3200 µg/L should be diluted with Saline Solution and re-assayed. The result is multiplied by the appropriate dilution factor. Results below 100 µg/L should be reported as ≤ 100 µg/L. There is no dose-hook effect up to 100,000 µg/L.

Thrombolyzer application: The lower limit of detection (defined as mean of the blank + 3SD) is 0.10 mg/L (100 µg/L). The assay range is 0.15-14.4 mg/L (150-14400 µg/L). Samples above 2.0 mg/L (2000 µg/L) will automatically be re-assayed with the follow-up test, D-dimer High. Samples containing > 14.4 mg/L (14400 µg/L) should be diluted 1:10 with Saline Solution and re-assayed. The result is multiplied by the dilution factor 10. Results below 0.15 mg/L (150 µg/L) should be reported as ≤ 0.15 mg/L (150 µg/L). There is no dose-hook effect up to 100 mg/L (100,000 µg/L). (See application sheet, part number 646-097, for use on Behnk Thrombolyzer line of instruments).

F. Specificity

The monoclonal antibody used in this device, MA-8D3, is specific for D-dimer by virtue of the screening method used for hybridoma selection.¹ A hybridoma, secreting antibodies with a 1000-fold greater affinity for purified D-dimer and fragment D of fibrin, than for native fibrinogen, was selected.⁴ Studies with the Auto•Dimer[®] demonstrate over one hundred fold higher reactivity with cross-linked fibrin degradation products than fibrinogen degradation products.

XIV. REFERENCES

1. Declerck, P., *et al.* Fibrinolytic response and fibrin fragment D-dimer levels in patients with deep vein thrombosis. *Thrombosis and Haemostasis* 58, 1024-1029, 1987.
2. Lindahl, T., *et al.* Clinical evaluation of a diagnostic strategy for deep venous thrombosis with exclusion by low plasma levels of fibrin degradation product D-dimer. *Scand. J. Clin. Lab. Invest.* 58, 307-316, 1998.
3. Hansson, P.O., *et al.* Can laboratory testing improve screening strategies for deep vein thrombosis at an emergency unit? *J. Intern. Med.* 235, 143-151, 1994.
4. Holvoet, P., *et al.* Binding properties of monoclonal antibodies against human fragment D-dimer of cross-linked fibrin to human plasma clots in an in vivo model in rabbits. *Thrombosis and Haemostasis* 61, 307-313, 1989.
5. Ballegeer, V., *et al.* Fibrinolytic response to venous occlusion and fibrin fragment D-dimer levels in normal and complicated pregnancy. *Thrombosis and Haemostasis* 58, 1030-1032, 1987.

6. Dempfle, C-E., *et al.* The Fibrin Assay Comparison Trial (FACT). Evaluation of 23 Quantitative D-dimer assays as basis for the development of D-dimer calibrators. *Thrombosis and Haemostasis* 85, 671-8, 2001.

XV. KEY GUIDE TO SYMBOLS



Use by



Lot



Catalogue number



Manufacturer



For *in vitro* diagnostic use



Store at 2-8°C



Consult accompanying documents



Biological risks

Manufactured by:
Trinity Biotech plc,
IDA Business Park,
Bray,
Co. Wicklow,
Ireland.
Tel: (353) 1 276 9800,
Fax: (353) 1 276 9888,
Web: www.trinitybiotech.com

Distributed in North America by:
DiaPharma Group, Inc.
To Order: 1-800-526-5224
Tech Support: 1-800-447-3846
Fax: (513) 860-9635
E-mail: info@diapharma.com
Web: www.diapharma.com