

UMA CO., LTD.

2-19-6 Yokosuka

Matsudo, Chiba, Japan

**MEASURE D-Dimer**

Reagent for determination of Fibrin Degradation Product

Latex Immunoturbidimetric Method

2 - 8°C

IVD *In vitro* Diagnostics**QUALITY MANAGEMENT SYSTEM (BY TUV)*** **DO NOT** freeze

18 months/block from light

ISO 13485:2016**1. PURPOSE OF USE**

Providing a quantitative in vitro assay for the fibrin degradation products (D-dimer) concentration in plasma.

2. GENERAL INSTRUCTION

- For in vitro diagnostics use only.
- Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.
- For guaranteed results, usage of this product must comply with the instruction in this manual.
- If you use automatic analyzers, follow their instructions carefully.

SUMMARY

D-dimer assays use mono- or polyspecific antibodies against D-dimer to provide quantitative or qualitative data on the concentration of D-dimer in whole blood or plasma. D-dimer is the product of lysis of cross-linked fibrin and the levels of D-dimer are increased in patients with acute VTE. However, the test is nonspecific because the level of D-dimer can be increased in a variety of other conditions, including malignancy, inflammatory conditions, and infections. Therefore the D-dimer assay is most useful as a tool to rule out suspected DVT.

D-dimer assays have two principal limitations: (1) a positive test result is nonspecific and should not be used as the sole criterion for diagnosis of VTE, and (2) numerous test kits are available that have different sensitivities for VTE. Thus D-dimer results are not interchangeable between kits. D-dimer assays employ different standards with some using fibrinogen and others using D-dimer. This results in differences in reporting because laboratory cut-offs depend on which standard is used. This has led to confusion among clinicians regarding the use of D-dimer assays. Further, the use of an insensitive D-dimer assay to rule out VTE could result in omission of required diagnostic testing, thereby placing patients at risk for PE and death.

The optimal setting for use of a D-dimer assay is in the assessment of patients with a low clinical pretest probability of VTE. The combination of a low pretest probability (determined using a validated scoring system) and a negative result with a validated D-dimer assay rules out the diagnosis of acute VTE, obviating the need for additional testing. Evaluation of the levels of D-dimer may be of value in patients with suspected recurrent VTE, and it may assist in decision-making about optimal duration of anticoagulation.

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water
- Micropipet and other basic laboratory equipment.
- D-Dimer Calibrator Set and D-Dimer Control Set

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Glycine buffer.

Reagent R-1 ready to use

- Reagent R-2: Mouse monoclonal anti-human D-dimer antibody latex solution

Reagent R-2 ready to use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrators (separately sold): Put 1 mL of purified water to each vial of calibrators (Level 1 - Level 6); leave at room temperature and sometimes mix for 40 minutes before use. After reconstituting, calibrators can be used without dilution.

- Controls (separately sold): Put 0.5 mL of purified water to each vial of control (Low - High); leave at room temperature and sometimes mix for 40 minutes before use. After reconstituting, controls can be used without dilution.

- Always use D-Dimer Calibrator Set for calibration.

5. SAMPLE PREPARATION & STORAGE

- Citrate plasma and lithium heparin plasma may also be used. Unlike when using citrated plasma, there is no sample dilution with heparin tubes. Therefore the D-Dimer values in heparin plasma are on average 16 - 20% higher over the entire measuring range.

- Analyze sample soon after collection. In case, it could not be analyzed soon, store sample 2 - 8°C.

- When using frozen samples, thaw them in room temperature and determine after mixing it well (transparent solution) before use.

- Stability:

- 1 day at 2 - 8°C
- 30 days at < -20°C

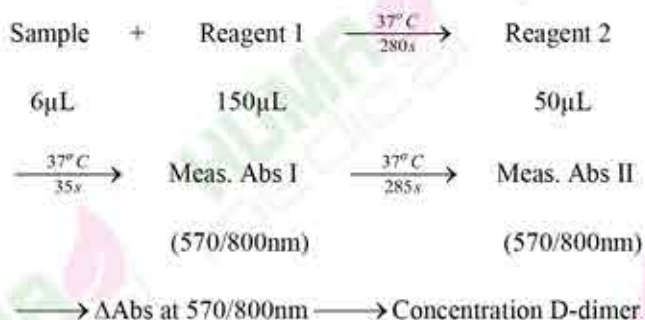
- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

D-dimer in patient samples develops a reaction with anti-human D-Dimer mouse monoclonal antibody-sensitized latex and turbidity increase when coagulation occurs. D-dimer in patient samples can be determined by measuring the variation of turbidity by spectrophotometer.

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.



Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD.** for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank
- Plot a calibration curve D-dimer = $f(\Delta\text{Abs})$
- Calculate D-dimer in specimen using the curve
(doing same procedure for Controls)

Unit conversion

$$\mu\text{g/mL} \times 1000 = \text{ng/mL}$$

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an D-dimer concentration range in serum/plasma of 0.5 - 50 $\mu\text{g/mL}$.

- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB) = 0.15 $\mu\text{g/mL}$

Limit of Detection (LoD) = 0.20 $\mu\text{g/mL}$

Limit of Quantitation (LoQ) = 0.50 $\mu\text{g/mL}$

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: When measuring known concentration of control sera (D-dimer 0.0 $\mu\text{g/mL}$ and 0.5 $\mu\text{g/mL}$) for 5 times simultaneously, mean value of 0.5 $\mu\text{g/mL}$ minus 2SD is mean value of 0.0 $\mu\text{g/mL}$ plus 2SD.

- Accuracy: When measuring a control sample, the result is within $\pm 10\%$ of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guideline EP5-A2 with repeatability, reproducibility and total precision (1 aliquot per run, 2 run per day, 20 days). The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 5% and Total Precision is less than 10%.

<i>Repeatability</i>	<i>Mean</i> <i>µg/mL</i>	<i>SD</i> <i>µg/mL</i>	<i>CV</i> <i>%</i>
D-dimer Control Low	1.14	0.03	2.65
D-dimer Control High	12.98	0.17	1.32

<i>Reproducibility</i>	<i>Mean</i> <i>µg/mL</i>	<i>SD</i> <i>µg/mL</i>	<i>CV</i> <i>%</i>
D-dimer Control Low	1.14	0.04	3.49
D-dimer Control High	12.98	0.30	2.33

<i>Total precision</i>	<i>Mean</i> <i>µg/mL</i>	<i>SD</i> <i>µg/mL</i>	<i>CV</i> <i>%</i>
D-dimer Control Low	1.14	0.05	3.96
D-dimer Control High	12.98	0.33	2.51

e. Correlation Test

Company A (same principle)

Regression equation: $y = 1.0072x - 1.0291$ ($n = 67$)

Correlation coefficient: $r = 0.994$

10. EXPECTED VALUES

Less than 1.0 µg/mL

There may be reactions or interfering reactions with non-target substances. If plasma with difficult sampling was used, falsely high values may be obtained. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated bilirubin concentration up to 21 mg/dL, free bilirubin concentration up to 18 mg/dL.

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL.

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 1420 FTU

- Rheumatoid Factor (RF): No significant interference of concentration up to 550 U/mL.

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL**Handling**

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

• Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

• Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ For Hitachi Model

Calculation Method		Two point
Temperature		37°C
Volume (μL)	Specimen	6
	R1	150
	R2	50
Wavelength (nm)	Main	570
	Sub	800
Measurement (cycle)	Point 1	10
	Point 2	18
	Point 3	34
Calibration type		Spline
Unit		μg/mL

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.
- Prepare the calibration curve on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11D011B	1x30mL; 1x10mL	155	270
11D011B2	2x30mL; 2x10mL	310	540
11D001B	5x30mL; 5x10mL	775	1350
11D011F	2x30mL; 2x110mL; D-dimer Calibrator Set 1mLx6; D-dimer Control Set 0.5mLx2	310	540
11D601B	1x30mL; 1x10mL; D-dimer Calibrator Set 1mL x 6	155	270

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series;

Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;...

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. Deborah Siegal, Wendy Lim, in Hematology (Seventh Edition), 2018
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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