MAGLUMI D-dimer (CLIA)

REF 130206008M

$\sum$ 100

Shenzhen New Industries Biomedical Engineering Co., Ltd
4F,Wearnes Tech Bldg,
Science & Industry Park,
Nanshan,Shenzhen,518057CHINA
Tel. + 86-755-86028224
Fax.+ 86 755 26654850

EC REP

Lotus Global Co., Ltd
15 Alexandra Road
London
NW8 0DP
UK
Tel. + 44-20-75868010
Fax.+ 44-20-79006187

CE

<u>FOR PROFESSIONAL USE ONLY</u>
Store at 2...8 °C

⚠ <u>COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING</u>

en

SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in Europe
▲	Manufacturer
📖	Attention. See Instructions For Use
CONT	Contents of kit
IVD	In vitro diagnostic medical device (In vitro diagnostic use)
LOT	Lot number
REF	Catalogue Code
⧖	Expiry date (Use by…)
2°C–8°C	Temperature limitation (store at 2...8 °C)
$\sum$	Number of tests
☼	Keep away from sunlight
↑↑	Keep upright
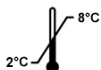
INTENDED USE

The kit has been designed for the quantitative determination of D-dimer in human **plasma (suggested)**.

The method can be used for samples over the range of 0-100 μg FEU/ml.

The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

SUMMARY AND EXPLANATION OF THE TEST

D-dimer is a fibrin degradation product (or FDP), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two crosslinked D fragments of the fibrinogen protein.

D-dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients suspected of thrombotic disorders. While a negative result practically rules out thrombosis, a positive result can indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thromboembolic disease where the probability is low. In addition, it is used in the diagnosis of the blood disorder disseminated intravascular coagulation.

D-dimers are not normally present in human blood plasma, except when the coagulation system has been activated, for instance because of the presence of thrombosis or disseminated intravascular coagulation. The D-dimer assay depends on the binding of a monoclonal antibody to a particular epitope on the D-dimer fragment. Several detection kits are commercially available; all of them rely on a different monoclonal antibody against D-dimer. Of some of these it is known to which area on the D-dimer the antibody binds. The binding of the antibody is then measured quantitatively by one of various laboratory methods

D-dimer testing is of clinical use when there is a suspicion of deep venous thrombosis (DVT) or pulmonary embolism (PE). In patients suspected of disseminated intravascular coagulation (DIC), D-dimers may aid in the diagnosis.

For DVT and PE, there are various scoring systems that are used to determine the a priori clinical probability of these diseases; the best-known were introduced by Wells et al.

PRINCIPLE OF THE TEST

Sandwich immunoluminometric assay;

Use an anti-D-dimer monoclonal antibody to label ABEI and use another monoclonal antibody to label FITC. Sample, Calibrator or Control with ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming a sandwich; After sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of D-dimer present in controls or samples.

CONT **KIT COMPONENTS**

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2% (W/V), 0.2%NaN$_3$, coated with sheep anti- FITC polyclonal antibody.	2.5ml
Calibrator Low: bovine serum, 0.2%NaN$_3$.	2.5ml
Calibrator High: bovine serum, 0.2%NaN$_3$	2.5ml

FITC Label: anti-D-dimer monoclonal antibody labeled FITC, contains BSA, 0.2%NaN$_3$.	6.5ml
ABEI Label: anti-D-dimer monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN$_3$.	6.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2% NaN$_3$. (target value refer to Quality Control Information date sheet)	2.0ml

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8 ℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.

- Keep upright for storage.

- Keep away from direct sunlight.

CALIBRATION AND TRACEABILITY

1)Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.
Calibrators in the Reagent Kit are from Fitzgerald.

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lots (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

SPECIMEN COLLECTION AND PREPARATION

- Collect samples using standard procedures.
- Sample material: **plasma (suggested)**.
- Store at 2-8℃: 12 hours.

- For longer storage periods: freeze to below - 20℃.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).

Serum

- Elbow vein blood 5ml in the tube, centrifugation at room temperature, serum was separated and stored at 2 ℃-8 ℃
- Serum samples were stable for 12 hours at 2-8 ℃. If preserved more than 12 hours, please seal the samples, -20 ℃ can be stored for 30 days,
- Avoid repeated freezing and thawing twice above.

Plasma:

- Elbow vein blood 5ml in the tube and then add EDTA anticoagulant, centrifuged and separated plasma, stored at 2-8 ℃.
- Plasma was stable at 2-8 ℃ for 12 hours. If pre than 12 hours, please seal the samples, -20 ℃ can be stored for 30 days.
- Avoid repeated freezing and thawing twice above.

Vacuum Tubes

(a) Blank tubes or EDTA tubes are recommended type for collecting samples.
(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service, for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

• Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

IVD

• For use in *IN-VITRO* diagnostic procedures only.
• Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

• The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
• All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p.2143). A minimum of one hour at 121 ℃ is basically patient considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
• It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
• This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
• Safety data sheets are available on request.

Handling Precautions

• Do not use reagent kits beyond the expiration date.
• Do not mix reagents from different reagent kits.
• Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
• For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
• To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
• Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
• For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

20μl	Sample, calibrator or controls
+40μl	ABEI label
+40μl	FITC label
+20μl	Nano magnetic microbeads
10 min	Incubation
400μl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.
Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

• Observe quality control guidelines for medical laboratories
• Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results.
Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

2) Interfering Substances

No interference with test results is seen by concentrations of hemoglobin≤500 mg/dl, jaundice bilirubin≤30 mg/dl, rheumatoid factor≤100 IU/ml, or heparin≤100 IU/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

4) High-Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the MAGLUMI D-dimer assay, no high dose hook effect was observed when samples containing up to 50 μg FEU/ml.

RESULTS

1) Calculation of Results

• The analyzer automatically calculates the D-dimer concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve

procedure. The results are expressed in μg FEU/ml. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were：< 0.5 μg FEU/ml.
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision

Control	Mean(μg FEU/ml)	SD(μg FEU/ml)	CV%
Level 1	1.12	0.04	3.60
Level 2	5.08	0.18	3.54
Level 3	9.78	0.32	3.27

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision

Control	Mean(μg FEU/ml)	SD(μg FEU/ml)	CV%
Level 1	1.38	0.06	5.55
Level 2	5.34	0.28	5.24
Level 3	9.66	0.56	5.80

2) Analytical Sensitivity

The sensitivity is defined as the concentration of D-dimer equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.03 μg FEU/ml.

3) Specificity

The specificity of the D-dimer assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
Troponin I	50μg/ml	1%

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
4.9 μg FEU/ml	5μg FEU/ml	102%

5) Linearity

Use D-dimer calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration μg FEU/ml	Absolute linear correlation coefficient (r)
A	0.0	
B	0.5	r=0.9860
C	2	
D	10	
E	25	
F	100	

(6)Method comparison

A comparison of MAGLUMI D-dimer(y) with a commercially available D-dimer(x) using clinical samples gave the following correlations (μg FEU/ml):
Linear regression
$y=0.96x+0.3$
$r=0.960$
$S_{y.x}=2.1$

Number of samples measured:200
The sample concentrations were between 0.4-8 μg FEU/ml

REFERENCES

1. Freyburger G. Trillaud H.Labrouche S. et al. D-dimer. Strategy inthrombosis exclusion aglod standard study in 100 patients suspected of deep vein thrombosis or pulmonary embolism; 8 DD methods compared［J］. Thromb Haemost，1998, 79: 3237.

2. Lee AJ,Fowkes GR, Lowe GD,et al. Determinants of fibrin D-dimer in the Edinburgh Artery Study［J］. Arteriosder Thromb Vasc Biol, 1995, 15(2):1094.

3. TIMI IIIB investigators. Effects of tissue plasminogen activators and a comparison of early invasive and conservative strategy in unstable angina and non-Q wave myocardial infarction: Results of the TIMI ⅢB trial [J]. Circulation, 1994,89(8):1545.

4. Carr JM. Mckinney M. Mc Donagh J.et al. Diagnosis of disseminated intravascular coagulation: role of D-dimer [J]. Am J Clin Pathol，1989，91:280.

5. Pagana, K. D. & Pagana, T. J. (© 2007). Mosby's Diagnostic and Laboratory Test Reference 8th Edition: Mosby, Inc., Saint Louis, MO. Pp 347-348.

6. Wu, A. (© 2006). Tietz Clinical Guide to Laboratory Tests, 4th Edition: Saunders Elsevier, St. Louis, MO. Pp 332-333.

7. Thomas, Clayton L., Editor (1997). Tabers Cyclopedic Medical Dictionary. F.A. Davis Company, Philadelphia, PA [18th Edition].

8. Pagana, Kathleen D. & Pagana, Timothy J. (2001). Mosby's Diagnostic and Laboratory Test Reference 5th Edition: Mosby, Inc., Saint Louis, MO.