D-DIMER Microlisa

Microwell ELISA Immunoassay for the Quantitative Detection of D-Dimer in Human Plasma

1. INTRODUCTION

D-Dimer is generated as a specific soluble degradation product during fibrinolysis. Elevated D-Dimer levels are found in cases of disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism (PE) but other circumstances may also lead to high D-Dimer levels such as old age, pregnancy, cancer, liver disease and infection. The ELISA system has a higher sensitivity when compared with latex agglutination tests thus giving an improved aid in the diagnosis for the exclusion of venous thromboembolism (VTE).

2. INTENDED USE

D-Dimer Microlisa is designed for in-vitro quantitative determination of D-Dimer in human plasma.

3. PRINCIPLE

D-Dimer Microlisa test is an enzyme immunoassay based on sandwich ELISA. Anti-D-Dimer antibodies has been coated onto the wells of the microwell plate provided. Plasma sample including standards are pipetted into these wells. During the first incubation, human D-Dimer antigen from samples and coated antibody is react and after washing the enzyme conjugate (anti-D-Dimer antibody HRPO) is added after incubation and washing to remove all the unbounded enzyme conjugate. A substrate solution which acts on the bound enzyme is added to induce a colured reaction product. The intensity of this coloured product is directly proportional to the concentration of D-Dimer present in the samples.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2016.

Manufactured By	*In vitro* diagnostic medical device
No. of tests	See Instruction for use
Lot Number Batch Number	Temperature Limitation
Manufacturing Date	Caution See instruction for use
Expiry Date	Catalogue Number
Do not use if package is damaged	Keep away from sunlight
Keep Dry	

5. PACK SIZE

- 96 Tests

6. COMPONENTS IN EACH PCT QUANTI MICROLISA KIT (96 TESTS)

Store all components at 2-8°C when not in use. Expiry date on the kit indicates that beyound which the kit should not be used.

D-Dimer Microlisa Strip Plates	Breakway microwells pre-coated with anti-D-Dimer antibodies in a pouch with dessicant.
Assay Diluent	Ready to use, buffer containg protein stabilizers and antimicrobial agents as preservatives.
Enzyme Conjugate	Ready to use, containing anti-D-Dimer antibodies labelled with HRPO with preservatives.
Wash Buffer Concentrate (25x)	PBS with surfactant. Dilute 1:25 with distilled water before use.
TMB Substrate	To be diluted with TMB diluent before use.
TMB Diluent	Buffer solution containing H_2O_2 with preservative
Standard-1	Ready to use, 0 ng/ml of D-Dimer with preservatives.
Standard-2	Ready to use, 100 ng/ml of D-Dimer with preservatives.
Standard-3	Ready to use, 400 ng/ml of D-Dimer with preservatives.
Standard-4	Ready to use, 1500 ng/ml of D-Dimer with preservatives.
Standard-5	Ready to use, 4000 ng/ml of D-Dimer with preservatives.
Standard-6	Ready to use, 10000 ng/ml of D-Dimer with preservatives.
Stop Solution	Ready to use, 1N sulfuric acid
Microwell Frame	Plastic frame for Microwells
Clamp & Rod	For sealing microwell pouch after use
Plate Sealers	Adhesive backed sheets for sealing microwell plate/strips.

7. STORAGE AND STABILITY

Store the kit & its component at 2-8°C. Expiry date on the kit indicates the date beyond which kit should not be used.

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- Micropipettes and microtips.
- ELISA Reader
- Distilled or deionized water
- Graduated Cylinders, for reagent dilution
- Sodium hypochlorite solution
- Paper towels or absorbent tissue
- Timer
- Microplate washer
- Incubator 37°C
- Vortex Mixer
- Disposable gloves
- Glassware

9. SPECIMEN COLLECTION & PREPARATION

1. Only human plasma samples (citrate plasma) should be used for the test. While preparing plasma samples, remove the plasma form the cells as soon as possible to avoid hemolysis. Fresh plasma samples are preferred.
2. Specimens should be free of microbial contamination and may be stored at 2-8°C for 3 days, or frozen at -20°C or lower for 30 days. Avoid repeated freezing and thawing.
3. Use of heat inactivated, icteric hyperlipemic and hemolyzed samples should be avoided as may give erroneous results.
4. Do not use samples containing sodium azide.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

D-Dimer Microlisa test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. If a centrifuge is available, the sample should be centrifuged at 10,000 rpm for 15 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

⚠ **CAUTION:** THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. STANDARDS & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves is RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.

3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant before disposal or by autoclaving at 121°C at 15psi for 60 min. They should be disposed off in accordance with established safety procedures and guidelines.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. ELISA Reader & micropipettes used in testing should be calibrated at regular interval to ensure accurate results.

12. PRECAUTIONS FOR USE

Optimal assay performance requires strict adherence to the assay procedure described in the manual.

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Bring all the reagents & samples to room temperature (20-30°C) before use.
3. Do not combine reagents from different batches, as they are optimised for individual batch to give best results.
4. Avoid microbial contamination of reagents. The use of sterile disposable tips is recommended while removing aliquots from reagent bottles.
5. Due to interchange of caps the reagents may get contaminated. Care should be taken while handling the reagents to avoid contamination of any sort.
6. Use freshly collected, clean plasma samples for assay. Try to avoid turbid, lipemic serum or plasma samples.
7. Use a separate tip for each sample and then discard it as biohazardous waste.
8. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
9. Do not allow microwells to dry once the assay has started.
10. Run all six standards in each assay to evaluate validity of the kit.
11. Incubation time should not vary by more than $\pm$ 1 min.
12. Prevent evaporation during sample incubation by covering the strips with strip sealer. Remove sealer before washing.
13. Distilled or deionised water must be used for wash buffer preparation.
14. Thorough washing of the wells is critical to the performance of the assay. Overflowing of reagents or washing to adjacent wells must be prevented during washing, which may lead to incorrect results due to carry over effect.
15. Take care while preparing working substrate solution as Bottle of TMB Substrate & TMB Diluent are of same size.
16. Prepare working substrate solution just 10 minutes prior to adding in the wells.
17. Use separate tips for TMB Substrate and TMB diluent.
18. Avoid strong light exposure during the assay.
19. Ensure that the microwell strips are levelled in the strip holder. Before reading, wipe the bottom of the microwell strips carefully with soft, absorbent tissue to remove any moisture.
20. In case of any doubt, the run should be repeated.

13. PREPARATION OF REAGENTS

Prepare the following reagents just before or during assay procedure. Reagents and samples should be at room temperature (20-30°C) before beginning the assay. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre-warm the incubator at 37°C.

- **D-Dimer Microlisa strips :**
  Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the microwell strips.

a. Break-off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that six standards should be included in each run.

b. **Unused wells should be stored at 2-8°C, with dessicant in aluminium pouch with clamp & rod. Microwells are stable for 30 days at 2-8°C from the date of opening of sealed pouch, when stored with desiccant along with clamp & rod.**

Caution: Handle microwell strip with care. Do not touch the bottom exterior surface of the wells.

- **Preparation of working Wash Buffer:**

a) Check the wash buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.

b) Prepare at least 50 ml (2 ml concentrated buffer with 48 ml water) of buffer for each strip used. Mix well before use.

c) Mix 30 ml of 25x wash buffer concentrate with 720 ml. of distilled or deionized water. **Working wash buffer is stable for 2 months when stored at 2-8°C.**

- **Preparation of working substrate solution :**

Mix TMB substrate and TMB Diluent in 1:1 ratio to prepare working substrate.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
No. of Wells	8	16	24	32	40	48	56	64	72	80	88	96
TMB Substrate (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0
TMB Diluent (ml)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0

Do not store working substrate. Prepare a fresh dilution for each assay in a clean plastic/glass vessel. Determine the quantity of working substrate solution to be prepared from table. Mix solution thoroughly before use. Discard unused solution. A deep blue color present in the substrate solution indicates that the solution has been contaminated and must be discarded.

14. PROCEDURAL NOTES:

1. Material should not be used after the expiry date shown on the labels. Components and test specimen should be at room temperature (20-30°C) before testing begins. Return the reagents to 2-8°C after use.
2. All reagents must be mixed well before use.
3. To avoid contamination, do not touch the top or bottom of strips or edge of wells.
4. All pipetting steps should be performed with utmost care and accuracy. Cross contamination between reagents and samples will invalidate results.
5. Prevent evaporation during sample incubation by covering the strips with sealer; remove sealer before washing.
6. Routine maintenance of wash system is strongly recommended to prevent carry over from highly reactive specimens to non reactive specimens.

15. TEST PROCEDURE

The instructions of the procedure must be strictly followed.

The sequence of the procedure must be carefully followed. Arrange the standards and samples in a horizontal or vertical configuration. Configuration is dependent upon reader software. **It is recommended to include all six standards in each run.**

(i) Fit the stripholder with the required number of D-Dimer Microlisa coated microwell strips.

(ii) Add 25 µl of each standard and sample (citrate plasma) in respective wells.

(iii) Add 100 µl of Assay diluent in each wells. Throughly mix the plate by 30-40 gentle shaking.

(iv) Cover the plate and incubate in an incubator at 37°C ± 1°C for 20 minutes.

(v) Wash the wells 3 times with working wash solution (300-350 µl). After final wash, invert and tap the plate strongly against absorbent/ tissue paper.

(vi) Add 100 µl of enzyme conjugate to each well.

(vii) Cover the plate and incubate in an incubator at 37°C ± 1°C for 20 minutes.

(viii) Wash the wells 3 times with working wash solution (300-350 µl). After final wash, invert and tap the plate strongly against absorbent/ tissue paper.

(ix) Add 100 µl working substrate solution in each well.

(x) Incubate at room temperature (20-30°C) in dark for 15 minutes and do not expose to light.

(xi) Add 100 µl of stop solution to each well.

(xii) Read the absorbance at 450 & 630 nm within 15 minutes in ELISA reader.

16. SUMMARY OF PROCEDURE

Add Standards & samples		25 µl
Add Assay Diluent		100 µl
Cover the plate & incubate		20 minutes at 37°C
Wash		3 Cycles
Add Enzyme Conjugate		100 µl
Cover the plate & incubate		20 minutes at 37°C
Wash		3 Cycles
Prepare TMB Substrate		No of Strips: 1 2 3 4 5 6 7 8 9 10 11 12 TMB Substrate (ml): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 TMB Diluent (ml.): 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0
Add Substrate		100 µl
Incubate in dark		15 minutes at Room Temp.
Add Stop Solution		100 µl
Read Results		In ELISA Reader at 450 nm and 630 nm

17. CALCULATION OF RESULTS

1. Calculate the mean absorbance values for each set of standards and samples.
2. Construct a best fit curve by plotting the absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the absorbance value for each sample determine the corresponding concentration from the best fit curve.
4. Automated Method : The results have been calculated automatically using a point to point curve fit which is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the sample can be read directly from the best fit curve. Sample with concentrations higher than that of the highest standard have to be futher diluted 1:5 with Standard-1 or reported as > 10000 ng/ml. For the calculation of the concentration, this dilution factor has to be taken into account.
6. For subsequent run, once master curve has been established in an ELISA Reader, calculate the results with stored master curve and absorbance of 3 standards with necessary data analytics.

Important Note: QC data sheet is batch specific and can be downloaded from company web site; www.jmitra.co.in

18. INTERPRETATION OF RESULTS

D-Dimer concentration of the test sample is given in terms of ng/ml (FEU, Fibrinogen equival units).

The cut-off (reference value): Below 500 ng/ml.

19. PERFORMANCE CHARACTERISTICS

Precision

Intra-Assay: Within precision have been determined by testing 10 replicates of 3 different samples with D-Dimer concentration (low, medium and high value respectively) on the same lot on same day. The C.V (%) is < 10%.

Inter-Assay: Between precision have been determined by testing 10 replicates of 3 different samples with D-Dimer concentration (low, medium and high value respectively) in 10 different run at different time interval. The C.V (%) is <15%.

Linear Range :

D-Dimer Microlisa is linear between 25 ng/ml to 10000 ng/ml.

Analytical Sensitivity

The sensitivity is defined as being the lowest detectable concentration different from zero with a probability of 95%. The sensitivity of the D-Dimer Microlisa kit is 25 ng/ml.

Specificity

There was no significant interference with the D-Dimer measurement observed when other biomolecules; Hemoglobin, Bilirubin, Albumin and Triglyceride were added to the test specimen with much higher level in normal plasma.

Accuracy: The accuracy of D-Dimer Microlisa was detected with 25 clinical specimen and compared with reference immunoassay test. The co-relation co-efficient is 0.995.

20. LIMITATION OF THE TEST

1. Any improper handing of samples or modification of this test might influence the results.
2. Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give erroneous results.
3. No hook effect was observed in this test
4. No substances (drugs) are known to us, which have an influence to the measurement of D-Dimer in a sample.

21. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

22. REFERENCES

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23. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Standards curve out of validation limit	a) Incorrect temperature timing or pipetting	Check procedure & repeat assay
	b) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay
	c) Cross contamination of standards/ reagents	Pipette carefully and do not interchange caps. use separate tip for standards / reagents.
	d) Used components from different lots.	Do not use components from different lots as they are adjusted for each batch released.
	e) Expired Reagents	Check the kit expiry date. Use the kit with-in shelf life
	f) Use of non calibrated micropipette and/or ELISA Reader	Calibrate micropipette and ELISA Reader at defined interval.
2. No colour developed at the end of assay	a) Any one reagent has been added in wrong sequence.	Follow the procedure meticulously & repeat assay.
	b) Inactivated conjugate, improper storage	Check storage of enzyme conjugate and it should be free of any contamination.
	c) Microplate inactivated, due to improper storage	Keep unused strips in aluminium poly pouch with the dessicant pouch inside and proerly closed with clamp & rod.
	d) Inactivated substrate, improper storage or preparation	Use freshly prepared substrate solution. Recheck procedure, repeat assay.
	e) Omission of any step in test procedure	Follow the procedure meticulously & repeat assay.
	f) Incorrect temperature timing or pipetting	Check procedure & repeat assay.
	g) Improper preparation of reagents, error of dilution, improper mixing of reagents.	Check procedure & repeat assay.
3. Too much colour in all wells of the plate (high background)	a) Contaminated substrate	Check substrate (TMB Diluent) it should be colourless. If blue in colour then discard and use clean disposable container.
	b) Contaminated washing solution (1X). Poor quality of water used for diluting wash buffer conc.	Check the container and quality of water used for dilution. Use of glass distilled water is preferred.
	c) Over incubation of substrate and delay in addition of stop solution.	Follow the procedure meticulously.
	d) Insufficient washing. i) Washing not consistent	Check wash device, fill the well close to the top.
	ii) Filling volume not sufficient.	After washing, blot the microwells on absorbent
	iii) Insufficient no. of wash cycles.	tissue. Follow wash protocol meticulously
	iv) Contaminated wash device	
	e) Use of wash solution from other manufacturer.	Use only D-Dimer Microlisa wash solution.
	f) Working substrate not protected from light	Incubate the plate in dark after addition of substrate.
4. Poor reproducibility	a) Washing problems. b) Uncalibrated pipettes or tips not well fitted, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	c) Reagent & sera not at room temperature or not well mixed before use.	Equilibrate reagents to room temperature and mix thoroughly before use
	d) Too long time for addition of calibrators, samples or reagents, inconsistency in time intervals.	Develop consistent and uniform technique.
	e) Interference in optical pathway due to Air bubbles.	Clean or dry the bottom of microwells, check for bubbles and repeat the readings.
5. High O.D for Standards & Samples	Beside 3a, b, c, d, e, incorrect interpretation and calculation of final results.	Check the calculation part given in the instruction manual and correctly interpret.
6. Low O.D for Standards and samples	a) Inadequate addition of standards/ substrate/conjugate solution	Follow the test procedure meticulously & repeat assay.
	b) Kit expired, reagent of different kit used.	Check the expiry of the kit before use.
	d) Uncalibrated pipettes, improper pipetting.	Use only calibrated pipettes with well fitted tips & pipette carefully without bubbling.
	e) Deterioration of Enzyme conjugate	Check storage of Enzyme conjugate. It shall be stored at 2-8ºC.
	f) Stop solution is added before 15 minutes. Reaction terminated before 15 minutes.	Follow the test procedure meticulously.
	g) O.D. taken at incorrect wavelength.	Read O.D. values at 450 nm and 630 nm.

VER-01 R-00

in vitro diagnostic Reagent, not for medicinal use

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