



## QUANTITATIVE TURBIDIMETRIC IMMUNOASSAY FOR ESTIMATION OF D-DIMER IN HUMAN PLASMA

### SUMMARY

During coagulation sequence of reactions occurs in the body in response to variety of external and or internal stimuli. The enzymatic cascade reaction terminates in the conversion of FIBRINOGEN to FIBRIN, by the enzyme THROMBIN. The fibrin gel is then converted to a stable fibrin clot by thrombin activated Factor XIII.

Finally, the fibrin network is dissolved by the enzyme PLASMIN to generate cross-linked fibrin degradation products (XL FDP). D-Dimer comprising of two D fragments cross linked together, is the smallest plasmin resistant molecular unit present within the cross linked fibrin degradation products.

Detection of D-Dimer is invaluable as a diagnostic marker for thrombotic conditions such as DIC, DVT and PE. The role of the pretest clinical probability score and/or the D-Dimer concentration in the diagnostic management of deep venous thrombosis has been supported by several different studies. The strategy of combining clinical assessment with D-Dimer testing in the initial evaluation of outpatients with suspected deep venous thrombosis is a potentially powerful means to rule out the DVT rapidly and safely, without the need for ultrasonography.

D-Dimer levels can also be used to monitor thrombolytic therapy with t-PA and with streptokinase, thrombotic complications in pregnancy, acute myocardial infarction, sickle cell crisis, severe septic infections, liver disease, DIC accompanying snake bite and prognosis and response to therapy in cancer. D-Dimer testing has been evaluated as a predictive factor for recurrences of VTE after discontinuation of oral anticoagulant therapy.

**Quantia® D-Dimer** is a quantitative Immunoturbidimetric assay for the determination of D-Dimer in human plasma.

### REAGENT

1. **Quantia® D-Dimer Activation buffer (R1):** ready to use buffer.
2. **Quantia® D-Dimer latex reagent (R2):** A uniform suspension of polystyrene latex particles coated with mouse monoclonals Anti D-Dimer Antibody. The cross-linked fibrin degradation products, D-Dimer, D-Dimer E, and high molecular weight derivatives are all recognized by **Quantia® D-Dimer** latex reagent. No binding was found to the fibrinogen degradation products X, Y, D, and E to 20 mg/L or to fibrinogen upto 1000 mg/L.
3. **Calibrator:** Lyophilized preparation of D-Dimer positive plasma, which is equivalent to the stated amount of D-Dimer on ng/ml basis when hydrated appropriately.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

### REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial labels. Once opened the shelf life of the latex reagent and activation buffer is as described on its vial label provided it is not contaminated.
3. The reconstituted **Quantia® D-Dimer** calibrator is stable for 7 days at 2-8°C and 48 hours at 25-30°C (RT.).
4. The working reagent for **Quantia® D-Dimer** can be prepared by mixing R2 and R1 in the ratio 1:2.
5. The mixed stability of the working reagent (R1+ R2) is 7 days when stored at 2-8°C.

### PRINCIPLE

**Quantia® D-Dimer** is a turbidimetric immunoassay for the determination of D-Dimer and is based on the principle of agglutination reaction. The test specimen is mixed with **Quantia® D-Dimer** latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of D-Dimer in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at wavelength 630 nm. The increase in turbidity corresponds to the concentration of D-Dimer in the test specimen.

## NOTES

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Gently mix the **Quantia® D-Dimer** latex reagent well before use to disperse the latex particles uniformly to improve test performance.
6. The working reagent should be mixed gently.
7. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
8. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
9. Calibrators of different manufacturers must not be used with **Quantia® D-Dimer** reagents.
10. The calibration curve must be validated periodically with known controls.
11. The **Quantia® D-Dimer** assay is recommended for analyzers with cuvette mode. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
12. The procedure mentioned in this pack insert is based on a minimum reading volume of 400 µl (0.4 ml). In case of instruments where minimum volume required for reading absorbance is higher, use double the quantity of reagents and samples mentioned in the test procedure.
13. Do not use damaged or leaking reagents.

## SAMPLE COLLECTION AND PREPARATION

No special preparation of the patient is required prior to sample collection. Plasma samples are recommended for use with **Quantia® D-Dimer** test. Fresh EDTA, citrate or heparinised anticoagulated plasma specimens are suitable for performing the test.

Sample storage: 20-25°C - 8 hours  
2-8°C - 4 days  
Frozen (-20°C) - 2 months

Thaw frozen specimens at 37°C and centrifuge plasma before testing.

## ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 630 nm wavelength filters and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable/glass **semi micro cuvettes**.

**Note:** Though any filter between the wavelengths 600-660 nm can be used, optimum results are obtained with 630 nm wavelength filter.

## TEST PROCEDURE

Bring reagent and sample to room temperature before use.

Assay conditions;

|                      |                  |
|----------------------|------------------|
| Wavelength           | 630 nm           |
| Reaction Temperature | 37°C             |
| Cuvette              | 1 cm path length |

## Method for preparation of D-Dimer Calibration Curve

The **Quantia® D-Dimer** calibrator must be reconstituted exactly with 1.0 ml of distilled water, wait for 10 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for preparation of D-Dimer calibration curve. The Concentration (S) of D-Dimer in the reconstituted calibrator is as mentioned at the end of this package insert.

Dilute the calibrator serially as mentioned below for preparation of calibration curve.

| Test Tube No.                           | 1   | 2   | 3   | 4   |
|-----------------------------------------|-----|-----|-----|-----|
| Standard No.                            | D4  | D3  | D2  | D1  |
| Vol. of Saline in $\mu\text{l}$         | -   | 200 | 200 | 200 |
| Vol. of Calibrator (S) in $\mu\text{l}$ | 200 | 200 | 200 | 200 |
| Conc. of D-Dimer in ng/ml               | S   | S/2 | S/4 | S/8 |

The calibrator dilutions including the highest calibrator concentration and the calibration dilution with concentration  $\geq 400$  ng/ml **must** be used for the preparation of the calibration curve.

#### Test procedure for preparation of calibration curve

(Note: During calibration on instrument with programming facilities, increasing concentration of the standards must be used for preparing calibration curve.)

1. Zero the instrument with distilled water.
2. Pipette 400  $\mu\text{l}$  of **Quantia® D-Dimer** activation buffer (R1) and 100  $\mu\text{l}$  of **Quantia® D-Dimer** latex reagent (R2) in the measuring cuvette. Mix well and incubate for 5 minutes at  $37^\circ\text{C}$ .

OR

Pipette 500  $\mu\text{l}$  of the **Quantia® D-Dimer** working reagent in the measuring cuvette. Incubate for 5 minutes at  $37^\circ\text{C}$ .

3. Add 30  $\mu\text{l}$  of Standard D1, mix gently and start the stopwatch simultaneously.
4. Read absorbance (A1), **exactly at** ten seconds, and absorbance (A2) again at the end of **exactly 5** minutes.
5. Repeat steps No. 2-4 for each diluted calibrator (Standard D2 to D4) for preparing calibration curve.
6. Calculate  $\Delta A$  (A2-A1) for each standard (D1 to D4). Plot a graph of  $\Delta A$  versus concentration of **D-Dimer** on the graph paper provided with the kit.

"The calibration curve" so obtained is valid only for the same lot of **Quantia® D-Dimer** reagents.

#### Test procedure for specimen

For determination of **D-Dimer** concentration in the test specimen:

1. Follow steps 2 - 4 as mentioned in the above procedure for calibration curve using the test specimen in place of the calibrator.
2. Calculate  $\Delta A$  (A2-A1) for the test specimen.

#### VALIDATION CRITERIA

If the  $\Delta A$  of the test specimen is less than  $\Delta A$  obtained for the standard of highest concentration (D1) then the concentration of **D-Dimer** in the test specimen can be determined directly by interpolating  $\Delta A$  of the test specimen from the calibration curve.

If the A of the test specimen is higher than  $\Delta A$  of standard with highest concentration (D1) then the test has to be rerun by carrying out dilution of test specimen such as 1:10, 1:20 etc., till the A of the diluted test specimen is less than A of D1. Then proceed for calculations.

#### CALCULATIONS

1. Interpolate  $\Delta A$  of the diluted test specimen on the calibration curve and obtain the **D-Dimer concentration 'C'** of the diluted test specimen.
2. Multiply the **D-Dimer concentration 'C'** with the dilution factor (F) of the test specimen for obtaining the concentration of **D-Dimer** in the neat test specimen.

Concentration of **D-Dimer** in the neat test specimen in ng/ml =  $C \times F$ .

(Where 'F' is the dilution factor of the test specimen, for e.g. 10 for 1:10 dilution of test specimen and so on).

#### SPECIFIC PERFORMANCE CHARACTERISTICS

##### Measuring range

The **Quantia® D-Dimer** reagent has been designed to measure **D-Dimer** concentrations in the range 400 - 3200 ng/ml. The exact range is dependent on the calibrator value, which is lot specific. The **Quantia® D-Dimer** assay is linear between the measuring range under the described assay conditions. The linearity limit of the measuring range depends on the sample to reagent ratio as well as the analyzer used. It will be higher by decreasing the sample volume, though the detection limit of the assay will be proportionately decreased.

### Detection limit / Analytical Sensitivity

Detection limit: 400 ng/ml

The detection limit represents the lowest measurable **Quantia® D-Dimer** concentrations that can be distinguished from zero.

### Prozone limit

No prozone effect was observed upto a concentration of 10000 ng/ml of **D-Dimer**.

### Precision

| Intra-assay precision | n  | Mean (ng/ml) | SD    | CV (%) |
|-----------------------|----|--------------|-------|--------|
| Sample 1              | 10 | 3047         | 39.84 | 1.31   |
| Sample 2              | 10 | 1016         | 39.9  | 3.93   |
| Sample 3              | 10 | 253          | 12.09 | 4.78   |

| Inter-assay precision | n  | Mean (ng/ml) | SD    | CV (%) |
|-----------------------|----|--------------|-------|--------|
| Sample 1              | 10 | 3042         | 52.44 | 1.72   |
| Sample 2              | 10 | 1021         | 38.62 | 3.78   |
| Sample 3              | 10 | 256          | 15.58 | 6.09   |

### Interference

No interference was observed with:

| Interference factor | No interference upto |
|---------------------|----------------------|
| Glucose             | 500 mg/dl            |
| Albumin             | 10 g/dl              |
| Bilirubin           | 50 mg/dl             |
| Haemoglobin         | 500 mg/dl            |
| Triglycerides       | 1000 mg/dl           |

### REFERENCE VALUES

The reference values of **D-Dimer** in normal population with **Quantia® D-Dimer** were estimated to be < 200 ng/ml.

Each laboratory should define its own reference range for relevant population.

### REMARKS

1. Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results.
2. When  $\Delta A$  obtained for the test specimen is greater than the  $\Delta A$  of the standard with highest concentration then, it indicates that the concentration of **D-Dimer** in the test specimen is beyond the measuring range of the **Quantia® D-Dimer** assay. Such specimens should be rerun with further dilutions.
3. Markedly lipemic, hemolysed, and contaminated plasma samples could produce erroneous **D-Dimer** values.
4. Do not read results beyond two minutes.
5. It is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis.

### WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

### BIBLIOGRAPHY

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