D-DI

Tina-quant D-Dimer

cobas®

Specific proteins

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03001245 322	Tina-quant D-Dimer (100 tests)	System-ID 07 6458 2	COBAS INTEGRA 400 plus COBAS INTEGRA 800
11556495 216	D-Dimer Calibrator (1 × 0.5 mL)	System-ID 07 7985 7	
	Zero Standard (1 × 2.5 mL)		
11556509 216	D-Dimer Control I (2 × 0.5 mL) Control II (2 × 0.5 mL)	 System-ID 07 7984 9 System-ID 07 7983 0	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test D-DI, test ID 0-758

Intended use

In vitro test for the quantitative immunological determination of fibrin degradation products (D-Dimer and X-oligomers)[1,2] in human plasma on COBAS INTEGRA systems.
In conjunction with a non-high clinical probability assessment, a normal (< 0.5 µg FEU/mL) result excludes deep vein thrombosis (DVT) and pulmonary embolism (PE) with high sensitivity.

Summary

Thrombin converts fibrinogen to soluble fibrin by cleaving the fibrinopeptides A and B. The fibrin monomers polymerize spontaneously. Active factor XIII links two D-domains and generates a solid fibrin clot. A new plasmin-resistant antigenic determinant ("D-Dimer") is produced. Fragments containing D-Dimer are formed accordingly during the degradation of a fibrin clot by plasmin.

A large proportion of the fibrin degradation products consists of high molecular weight X-oligomers. The Tina-quant D-Dimer assay has a strong affinity for these high molecular weight degradation products. Only in vitro or during lysis therapy does complete degradation to D-Dimer molecules take place.

D-Dimer is a very sensitive marker for the activation of coagulation. When D-Dimer values below the cutoff are obtained, deep venous thrombosis (DVT) of the lower limb and pulmonary embolism (PE) can be excluded with high sensitivity.[3,4,5,6]

The evidence for the use of Tina-quant D-Dimer in exclusion diagnosis comes from prospective management studies.[7,8,9]

In one such study of 812 outpatients with symptoms of DVT, Schutgens et al. found that the combination of a non-high clinical probability score and a normal Tina-quant D-Dimer concentration allowed rule-out of DVT with a sensitivity of 99.3 % and a Negative Predictive Value (NPV) of 99.4 %.[7] This rule-out strategy was found to be very safe, with a failure rate of only 0.6 %. Only 1 of 176 patients with a non-high pretest probability and a normal D-Dimer developed thrombosis during the three month follow-up. In a study involving 202 outpatients with suspected PE, Leclerq et al found that PE could be ruled out by a normal Tina-quant D-Dimer result combined with a non-high clinical probability score, with a sensitivity of 100 %, an NPV of 100 % and a failure rate of 0 %.[9] Further supporting evidence comes from numerous other clinical studies.[10,11,12,13,14,15,16,17,18,19,20]

The D-Dimer result should not be used in isolation but in combination with a clinical probability assessment like the Wells score. DVT/PE should only be excluded on the basis of a low or moderate (non-high) clinical probability and a normal (< 0.5 µg FEU/mL) Tina-quant D-Dimer result.

It has been reported that patients with a distal DVT or a subsegmental/ peripheral PE may have a normal Tina-quant D-Dimer result.[21] The clinical relevance of such small(er) thrombi is unclear. The good results obtained in the management studies where patients were treated based on the Tina-quant D-Dimer result and then followed-up for 3 months suggest that these smaller thrombi do not result in adverse patient outcomes.[21]

In disseminated intravasal coagulation (DIC)/consumptive coagulopathy, fibrin degradation products are a sensitive marker. Monitoring the fibrin-specific degradation products can be used to

- confirm or refute a tentative diagnosis
- estimate the potential risk for patients with existing DIC
- monitor an initiated therapy

Apart from DVT, PE and DIC, D-Dimer may reflect other causes associated with fibrin formation such as trauma, pregnancy complications, malignant disease or vascular abnormalities. Elevated D-Dimer levels therefore have to be interpreted in the context of possible underlying diseases and clinical symptoms.[22,23,24]

Test principle

Particle enhanced immunoturbidimetric assay.

Latex particles of uniform size are coated with monoclonal antibodies ($F(ab')_2$ fragments) to the D-Dimer epitope. The antigen/antibody complexes produced by the addition of samples containing D-Dimer lead to an increase in the turbidity of the test reactants. The change of absorbance with time is dependent on the concentration of D-Dimer epitopes in the sample. The precipitate is determined turbidimetrically at 659 nm.

Reagents - working solutions

R1	TRIS[a]/HCl buffer: 370 mmol/L, pH 8.2; NaCl: 267 mmol/L
R2	Latex particles coated with monoclonal anti-human D-Dimer antibodies (mouse): 0.15 %

a) TRIS = Tris(hydroxymethyl)-aminomethane

R1 is in position A and R2 is in position B.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

COBAS INTEGRA 400 plus system

All new (not punctured) cassettes must be mixed for 10 minutes using the off-board mixing station before placing on-board the analyzer. All in-use cassettes have to be mixed daily before use for one (1) minute on the cassette mixer.

COBAS INTEGRA 800 system

The reagent is automatically mixed for 10 minutes after cassette puncture and for half a minute during Begin of Day.

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Citrated plasma.

Collect venous blood using standard sampling tubes for clotting tests; employ sterile 0.11 molar sodium citrate solution. Maintain a precise mixture of 1 + 9 for sodium citrate and blood.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all

available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

If necessary, pipette off the supernatant and store in a stoppered plastic tube.

Li-heparin plasma may also be used.[25] Unlike when using citrated tubes, there is no sample dilution with heparin tubes. Therefore D-Dimer values in heparin plasma are on average 19 % higher over the entire measuring range. However, by using adjusted calibrator and control values, identical values are measured in patient specimens with both sample materials.

CAUTION. To avoid erroneous patient values, we recommend that all D-Dimer measurements are performed uniformly in the laboratory from either citrated plasma or heparin plasma.

Stability:[26]	8 hours at 15-25 °C
	4 days at 2-8 °C
	6 months at (-15)-(-25) °C

Thaw frozen samples completely at 37 °C and then mix thoroughly. Leave to stand for 15 minutes at room temperature before use; then assay immediately. Once thawed, a sample may not be refrozen for coagulation analysis.

Use the samples undiluted.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic postdilution. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1/R2-S
Reaction direction	Increase
Wavelength A/B	659 nm
Calc. first/last	T_0/38
Unit	µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	90 µL	
R2	90 µL	
Sample	5 µL	10 µL
Total volume	195 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-R2-S
Reaction direction	Increase
Wavelength A/B	659 nm
Calc. first/last	T_0/53
Unit	µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	90 µL	
R2	90 µL	
Sample	5 µL	10 µL
Total volume	195 µL	

Calibration

Calibrator	D-Dimer Calibrator
Calibration dilution ratio	Manual dilution of D-Dimer calibrator with zero standard. Follow the instructions in the D-Dimer Calibrator package insert.
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Calibration interval	Each lot, every 6 months when using a single lot of reagent and as required following quality control procedures.

Calibrators must be placed from the highest concentration first, to the lowest last, on the CAL/QC rack.

Traceability: This method has been standardized against the ASSERACHROM D-Dimer method.[27]

Quality control

Reference range	D-Dimer Control I/II
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factors:	µg FEU*/mL = mg FEU/L
	µg FEU/mL × 1000 = ng FEU/mL

*FEU = Fibrinogen Equivalent Unit

Limitations - interference

Results just below the cutoff normal/pathological (0.5 µg FEU/mL) should be considered pathological if the sample is either highly turbid or has an intense red color.

Criterion: Recovery within ± 10 % of initial value.

Icterus:[28] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[28] No significant interference up to an H index of 300 (approximate hemoglobin concentration: 300 mg/dL or 186 µmol/L).

Lipemia (Intralipid):[28] No significant interference up to an L index of 600. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[29,30]

High concentrations of D-fragments, as can occur during lysis therapy, lead to depressed measurements.

In rare cases, immunoglobulins, particularly in samples from patients with myeloma, can give falsely high results.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.1-9.0 µg FEU/mL

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution (postdilution 1) or a 1:6 dilution (postdilution 2). Results from samples diluted by the rerun function are automatically multiplied by a factor of 3 (postdilution 1) or by a factor of 6 (postdilution 2).

Lower limits of measurement

Lower detection limit of the test

0.1 µg FEU/mL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values[31]

< 0.5 µg fibrinogen equivalent units/mL (µg FEU/mL)

The stated fibrinogen equivalent is based on the quantity of fibrinogen used in the preparation of the original Asserachrom standard.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (repeatability n = 21, intermediate precision n = 21). The following results were obtained:

Repeatability	**Mean**	**CV**
Level 1	0.27 µg FEU/mL	6.9 %
Level 2	2.88 µg FEU/mL	1.1 %

Intermediate precision	**Mean**	**CV**
Level 1	0.28 µg FEU/mL	6.8 %
Level 2	2.89 µg FEU/mL	1.1 %

Method comparison

D-Dimer values for human citrated plasma samples obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Tina-quant D-Dimer reagent (y) were compared to the same reagent on a Roche/Hitachi 717 analyzer (x).

Values ranged from 0.04 to 4.20 µg FEU/mL.

		Roche/Hitachi 717 analyzer
Sample size	(n)	126
Corr. coefficient	(r)	0.994
Lin. regression		y = 1.01x + 0.03 µg FEU/mL
Passing/Bablok[32]		y = 1.01x + 0.02 µg FEU/mL

Clinical performance in the exclusion of DVT

Tina-quant D-Dimer was used in a multicenter management study involving 812 outpatients with suspected DVT.[7] Using the Wells probability assessment score, patients were classified as having a high (> 3) or non-high (≤ 3) pretest probability of DVT. The Tina-quant D-Dimer test was then performed using a cutoff of 0.5 µg FEU/mL. Those patients having a normal (negative) D-Dimer test result and a non-high pretest probability had no further diagnostic testing and were followed up for 3 months for development of DVT. Only one of 176 such patients developed DVT during the follow-up period. The performance characteristics of the Tina-quant D-Dimer assay in conjunction with a non-high pretest probability is summarized below:

Sensitivity:	99.3 % (95 % CI: 96.4-100 %)
Negative Predictive Value:	99.4 % (95 % CI: 96.9-100 %)
Specificity:	45.8 % (95 % CI: 40.7-51 %)
Positive Predictive Value:	42.0 % (95 % CI: 36.8-47.3 %)
Failure Rate:	0.6 % (95 % CI: 0.02-3.1 %)

Clinical performance in the exclusion of PE

Tina-quant D-Dimer was used in a management study involving 202 patients with suspected PE.[9] Using the Wells clinical model for PE probability[33], patients were classified as having a low, moderate or high pretest probability of PE. The Tina-quant D-Dimer test was then performed using a cutoff of 0.5 µg FEU/mL. Those patients having a normal (negative) D-Dimer test result and a non-high (low or moderate) pretest probability had no further diagnostic testing and were followed up for 3 months for development of PE. No patient developed PE during the follow-up period. The performance characteristics of the Tina-quant D-Dimer assay in conjunction with a non-high pretest probability is summarized below:

Sensitivity:	100 % (95 % CI: 91.8-100 %)
Negative Predictive Value:	100 % (95 % CI: 94.4-100 %)
Specificity:	50.4 % (95 % CI: 41.4-59.4 %)
Positive Predictive Value:	40.5 % (95 % CI: 31.1-50.5 %)
Failure Rate:	0 % (95 % CI: 0.0-5.6 %)

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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