CLEARTEST® DIAGNOSTIK

MADE IN GERMANY

D-DIMER TEST

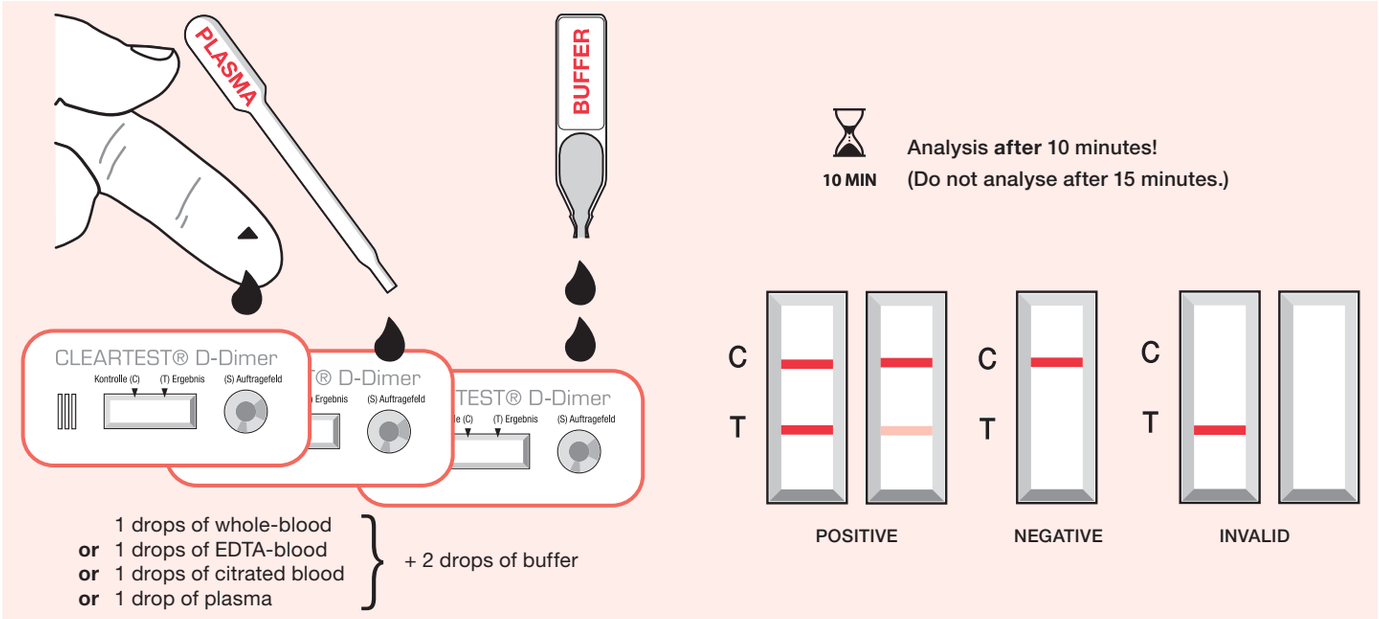
Rapid test for the detection of D-Dimer in whole-blood, EDTA-blood and citrated blood (new) and plasma

Only for professional in vitro diagnostics

IVD

INTENDED USE

The Cleartest® D-Dimer cassette test is a visual rapid test for the qualitative detection of D-Dimer in plasma, whole-blood, EDTA-blood and citrated blood (new).



This kit is an aid in the diagnosis of disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism, and is only suitable for in vitro diagnostic use by a professional practitioner.

ABSTRACT

D-Dimer tests were originally developed in the diagnosis of disseminated intravascular coagulation (DIC). Their benefit in the diagnosis of thrombo-embolic cases was recognised in the 1990s.

D-Dimer is a fibrin degradation product, a small fragment of protein, which is present in the blood after the degradation of a blood clot by way of fibrinolysis. During blood coagulation the fibrinogen is metabolised into fibrin through the activation of thrombin. Fibrin consists of D- and E-units. Splitting fibrin causes so-called D-Dimers.

The D-Dimer concentration can be determined with the aid of a blood test in order to support a thrombosis diagnostic. Since its implementation in the 1990s, this provision has become an important test for patients with suspected thrombotic disorders. Whilst a negative finding practically excludes thrombosis, a positive finding can indicate a thrombosis; however, it does not exclude the possibility of other possible diseases. Its main purpose therefore, is in connection with thromboembolic diseases if their probability is low.

D-Dimer tests are for clinical use if deep vein thrombosis (DVT) or pulmonary embolism (PE) is suspected. A D-Dimer test can support a diagnosis for patients with suspected disseminated intravascular coagulation (DIC).

TEST PRINCIPLE

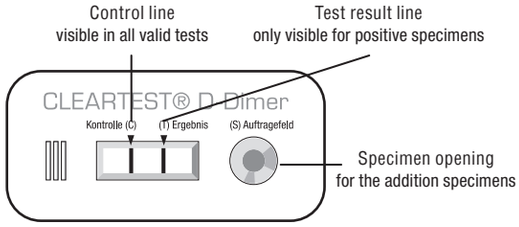
The Cleartest® D-Dimer cassette test is intended for the detection of D-Dimer in plasma, whole-blood, EDTA-blood and citrated blood (new). This information can be used by the doctor and patient for treatment.

The Cleartest® D-Dimer cassette test was designed for the detection of D-Dimer in plasma, whole-blood, EDTA-blood and citrated blood (new) by means of visual interpretation of the colour development in the test cassette of a sandwich immunoassay. The membrane has been coated with an antibody against D-Dimer in the test line region (T).

During the test the diluted specimen can react with a dyed conjugate (anti-D-Dimer antibody – gold conjugate), which is administered on the pad inside the test cassette. The mixture then moves chromatographically by way of a capillary action via the membrane. If D-Dimer is present in the specimen, a coloured line forms in the test line region (T) of the membrane with a specific antibody-antigen-conjugate complex. This complex consists of a coloured anti-D-Dimer antibody, D-Dimer from the specimen and antibody fixed to the membrane in the test line region (T).

On the other hand, the control region (C) will always exhibit a coloured line. To do this another antigen-antibody reaction is used. This control line serves as a procedure indicator of the kit's proper function. It shows that the test procedure has been executed correctly and that the specimen properly ran over the membrane.

A pronounced colour development in the test line region (T) exhibits a positive finding. Where there is no coloured line in the test line region (T) this exhibits a negative finding.



STORAGE AND STABILITY

The test kit can be stored at temperatures from +2 to +30°C throughout its shelf life.

WARNINGS

- Only suitable for in vitro diagnostic use by professional practitioners.
- The kit must not be used after expiration of the shelf life.
- Thoroughly read the instructions prior to use
- Do no use if the film bag shows signs of damage because the test is sensitive to moisture.
- Only open the film bag when you are ready to carry out the test.
- Do not use more than once.

- Food, drink and smoking are prohibited in the areas in which the specimen material or tests are processed.
- All patients‘ specimen material must be treated as potentially infectious. During the test, please observe all approved precautionary measures for handling biologically dangerous materials and follow the standard procedures for the proper disposal of specimen material.
- The diluting solution contains small amounts of Bromo-nitro-dioxane (0.05%).
- Do not pipette reagents by mouth.
- Do not spray any solution in the reaction area.
- Do not disturb the reaction area of the kit in order to prevent contamination.
- Protective clothing, such as laboratory coats, disposable gloves and eye protection, must be worn when testing specimen materials.
- Always store and transport the test cassette at 2–30°C (36–86°F).
- Moisture and high temperatures can influence the findings.

SUPPLIED REAGENTS AND MATERIALS

- Test cassettes
- Pipettes
- Single dropper with diluting buffer solution (PBS with 0.05% BND)
- 1 set of instructions

ADDITIONALLY REQUIRED MATERIALS

- Container to collect specimen (citrate tubes/EDTA tubes)
- Lancet
- Timer

SPECIMEN EXTRACTION AND HANDLING

- The Cleartest® D-Dimer cassette test can be carried out with whole-blood without any additives (from venipuncture or fingertip), EDTA-blood, citrated blood or plasma. Where the test is carried out with whole-blood it must be fresh.
- Separate the plasma as quickly as possible in order to prevent haemolysis. Please exclusively use clear, not haemolysed specimen material.
- The test should be carried out directly after the specimen has been taken. Do not leave a specimen sitting at room temperature for a long period of time.
- D-Dimers are very unstable molecules. Plasma specimen can only be stored at room temperature for 8 hours and chilled (+4°C) for 24 hours.
- Specimen must be brought up to room temperature prior to carrying out the test.
- If specimen material is to be sent, it must be packed in accordance with the statutory provisions for the transport of pathogens.

TEST PROCEDURE

Test cassette, buffer solution and the patient‘s specimen material must be brought up to room temperature (+20–30°C) prior to testing. Only open the film bag when everything is ready to carry out the test.

1 Remove the test cassette from the protective bag (bring the cassette to room temperature prior to opening the bag so that no moisture can deposit on the membrane). Label the test cassette with a patient or control number.

2 First administer 2 drops of blood (capillary, EDTA or citrated blood) or 1 drop of plasma (with the pipette supplied with the test) into the specimen openings and let it soak in, then add 2 drops of buffer solution. Ensure that no solution is sprayed into the reaction area.

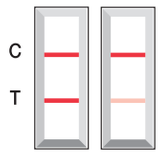
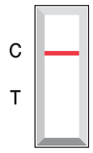
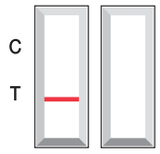
Should the test not have visibly started after 10 seconds, add a further drop of buffer.

Start timer!

3 Read off the findings exactly 10 minutes after adding the specimen.

Do not, under any circumstances, read off the findings after 15 minutes.

INTERPRETATION OF FINDINGS

	**POSITIVE FINDING:** In addition to the control line, there will also be a pronounced red-coloured line in the test line region. Note: The colour intensity of the lines can differ.
	**NEGATIVE FINDING:** A red-coloured line only appears in the control line region. A recognisable, red-coloured line is not visible in the test line region.
	**INVALID FINDING:** If there is not a control line in the C region, the test is inconclusive and must be deemed to be invalid. A missing control line can indicate an error in the test procedure or that the contents of the assay are not in order. Repeat the test with a new test cassette and pay particular attention to the instructions when doing so. If the problem remains, contact the manufacturer.

QUALITY CONTROL

The test contains an internal procedure control. A reddish control line in the control region (C region) of the membrane shows that the test has been carried out correctly.

Within the scope of good manufacturing practice (GMP), the application of external controls for detection of the test‘s proper function is recommended.

EXPECTED DATA

Increased D-Dimer concentrations indicate an active fibrinolysis and have been detected amongst patients with disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism. Such increased concentrations have however, also been detected after operations and injuries, where there is sickle cell anaemia, liver disease, severe infection, sepsis, inflammation, malignant tumour disease and amongst older people. The concentrations of D-Dimer also increase during normal pregnancy.

The Cleartest® D-Dimer cassette test‘s detection limit is 500 ng/ml. This is the amount at which it provides a positive finding.

CLINICAL PERFORMANCE CHARACTERISTICS

Precision

The test‘s precision has been determined in blind tests with control solutions. Controls with a D-Dimer concentration of 0 ng/ml provided a negative finding. Controls with a D-Dimer concentration of 500 ng/ml provided a positive finding.

Reproducibility

The reproducibility of the Cleartest® D-Dimer cassette test was confirmed in blind tests. They were carried out on different days. All specimens with D-Dimer concentrations of 0 ng/ml provided a negative finding. All specimens with D-Dimer concentrations of 500 ng/ml provided a positive finding.

Accuracy

The accuracy of the Cleartest® D-Dimer cassette test was compared with a commercially available test with a detection limit of 500 ng/ml (this test was validated against an ELISA test, the DIMERTEST, GOLD, EIA). The findings coincided in 95% of instances.

Specificity

The following substances did not produce any interferences with this test: Bilirubin up to 0.2 g/l, lipids up to 30 ng/l, serum protein up to 50 g/l, gamma globulin and haemoglobin 1 g/l.

A study of patients with rheumatoid arthritis did not exhibit cross-reactivity with the rheumatoid factor. The specificity of the Cleartest® D-Dimer sits at 92,31%. The sensitivity of the Cleartest® D-Dimer cassette test is 95,95 %.

Reference methode

		positive	negative	total
Cleartest® D-Dimer	positive	71	2	73
	negative	3	24	27
	total	74	26	100

LIMITS OF THE PROCEDURE

- A negative finding excludes the possibility of disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism by 99%.[2]
  Age-dependent D-dimer value, age * 10 applies from the age of 50. The D-dimer cut off shifts with increasing age. Pregnancy increases the probability of leg vein thrombosis by a factor of 4.
- A positive finding is not proof for confirmation of the aforementioned diseases. It shows a recently occurring deep vein thrombosis because after one week the D-Dimer concentration lowers to normal again.[3]
- As with all diagnostic procedures, the test and obtained findings must be used in conjunction with other information available to the practitioner.
- There is the possibility that the test does not provide any findings if the whole-blood specimens have a high viscosity or are stored for more than one day. In these instances, the test should be repeated with a new test and a fresh specimen from the same patient.
- Incorrect positive readings can have various causes: liver disease, inflammation, carcinoma, trauma, pregnancy, recent operations and advanced age.[2]
- Incorrect negative readings can occur where the specimen is either taken too early after thrombus formation or where the testing is delayed by a number of days. Furthermore, a treatment with anticoagulants prior to taking the specimen can cause a negative test finding because they prevent the increase of the thrombus.
- Increased D-Dimer values after a treatment with anticoagulants indicate a further existing risk of thrombosis.[4]

Incorrect negative findings

In the immunological D-Dimer test

Incorrect negative D-Dimer findings in immunological tests are frequently described in literature and therefore not unfamiliar. Alongside user error, there are various reasons for incorrect negative findings, which are subsequently presented:

Clinical probability

The sensitivity of immunological rapid tests for patients with mid to high probability of thrombosis (high Wells Score) is lower (negative forecast value = 85.7%) than for patients with lower clinical probability (lower Wells Score; negative forecast value = 99.5%). An ultrasound investigation independent of the rapid test findings is advised where there is mid and high probability.[3, 4]

Position of the thrombosis

Although the proximal vessels are implicated in the majority of deep vein thrombosis (DVT) cases, isolated calf vein thrombosis (distal vessels) accounts for about 20% of all deep vein thrombosis cases. The sensitivity of immunological tests is dependent on the position of the thrombosis and in proximal DVT accounts for a maximum of 94% and in distal DVT only a maximum of 67%.[6, 7] This can very likely be attributed to the fact that most cases of DVT begin in the distal vessels and therefore the number and/or the size of clots at the beginning of thrombus formation is/are small and subsequently the D-Dimer concentration is still too low.[4]

When to take specimens and carry out the test

If the specimen is taken too early after thrombus formation, the D-Dimer concentration will still be too low and will not present itself under the detection limit of the test system.[4]

If the test implementation is delayed or the specimen is inadequately stored, incorrect negative findings can subsequently occur. Fibrin derivatives are very unstable molecules. The investigation should preferably take place immediately after specimen extraction. Should this not be possible, the specimen can be stored at room temperature for 8 hours or 1 day chilled at +4°C.[2]

If the specimen is extracted too late after onset of the thromboembolic infarction, the D-Dimer concentration can fall below the detection limit. The D-Dimer concentration can already begin to decrease back to normal levels after one week.[1] In particular where the diagnosis by exclusion is acute pulmonary embolism, the D-Dimer concentration is clearly lower than for DVT and does not necessarily correlate with the severity of the pulmonary embolism. Thus the D-Dimer level in patients with massive pulmonary embolism can be just above the test-specific limit or even under.[1]

Anticoagulation therapy:

Scientific studies prove that treatment with anticoagulants swiftly decreases the D-Dimer concentration. That is the reason that a negative reading can occur if the specimen extraction is carried out after the beginning of the anticoagulant therapy.[4, 8, 9, 10]

Matrix effects

The so-called matrix effect is a general problem in immunological tests, which can lead to incorrect positive or incorrect negative findings. Matrix effects are the sum of disturbing effects of all components that appear in a specimen and influence the reading of target analytes. If the exact molecular cause of a disturbance is unknown, but can be related to composition of the specimen to be read, this is generally known as a matrix effect. As a result, the transition to the individual disturbing effects is seamless. Matrix effects can be triggered by „Anti-Animal Antibodies“, z.B. HAMAs (human anti-mouse antibodies), heterophile antibodies, endocrine disruptors (rheumatoid factors, albumin, complement, lysozyme) or influences on viscosity, pH value or the salt concentration.

Matrix effects are very difficult to prove, if at all. It could be demonstrated in an investigation for the provision of D-Dimers that HAMAs can be held responsible for the incorrect negative finding in a test system.[11]

Test system calibrators:

When interpreting the readings of D-Dimer tests, it should be considered that manufacturers use various calibrators in the absence of international reference standards and there is, therefore, no numerical agreement across the test procedures. This is also particularly applicable to the limit for exclusion of DVT or pulmonary embolism.[1]

The development of an international standard is impeded by the fact that the measured D-Dimer antigen is not a single analyte, but rather a mixture of fibrin derivatives of various molecular weights and different compositions.[1, 4]

Conclusion

A negative finding can contribute to excluding thromboembolic infarctions with a very high possibility.[1, 2] However, the diagnosis, not least due to the aforementioned reasons, should never depend alone on the findings of a rapid test. As with all diagnostic procedures, the findings as a result of the rapid test should always be used in conjunction with other information available to the practitioner, for example, the so-called „Wells Score“ for DVT or pulmonary embolism.[12] The findings of the D-Dimer test, in particular within the scope of the diagnosis of a DIC should have a place in the investigation of the so-called „DIC Score“(1) which has been developed by the International Society of Thrombosis and Haemostasis (ISTH).[13, 14]

LITERATURE

1 Dempfle, Carl-Erik (2005): Bestimmung des D-dimer-Antigens in der klinischen Routine (Determination of the D-Dimer antigen in the clinical routine); Deutsches Ärzteblatt Jg. 102, Booklet 7, 18 February 2005: A428-A432.

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12 Riddle and Wells (2004): Diagnosis of lower-extremity deep vein thrombosis in outpatients; Physical therapy, 84(8), 729-735

13 Constantinescu at al. (2007): Disseminated intravascular coagulation and a negative D-dimer test; The Netherlands journal of medicine, 65(10), 398-400

14 Bakhtiari et al. (2006): Prospective validation of the International Society of Thrombosis and Haemostasis scoring system for disseminated intravascular coagulation; Critical Care Medicine; 32, 2416-2421

SYMBOLE

REF	Item number		Temperature limit
	Operating instructions	LOT	Batch number
IVD	In-vitro-Diagnostikum		Expiration date
	Manufacturer		Content sufficient for <n> Tests
	Product for single use	CE	CE marked in accordance with the IVD directive 98/79/EG

ORDER INFORMATION

Cleartest® D-Dimer Test, 1 Test
REF **C3 0501-E**
PZN 06690833
4 052919 000837

Cleartest® D-Dimer Test, 5 Tests
REF **C3 0501-5**
PZN 06690856
4 052919 000806

Cleartest® D-Dimer Test, 10 Tests
REF **C3 0501-10**
PZN 06690862
4 052919 004088

CLEARTEST® CONTROL

For the internal quality control according to RiliBÄK, please check your IVD values regularly. The Cleartest® Control range provides you with reliable controls of German manufacturing with high precision and very easy application. For the appropriate rapid test in the declared range.

Type	Content	PZN	REF
Cleartest® Control HCG for pregnancy testing > 40 < 60 mIU/ml	1 vial	10629058	**K4 23101-1**
Cleartest® Control Humano Faecal for stool testing, >10 <20 ng/ml	1 vial	10629064	**K4 23102-1**
Cleartest® Control Troponin für troponin I, >1 <3 ng/ml	1 vial	10629070	**K4 23103-1**
Cleartest® Control D-Dimer positive for D-Dimer positive, 1 µg/ml	1 vial	10629087	**K4 23104-1**
Cleartest® Control D-Dimer negative for D-Dimer negative, 50 ng/ml	1 vial	10629101	**K4 23105-1**

IVD CE Created on: 2022-03-08 1-C3 0501-Eff-132-2-0003.1-2203

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