

INTENDED USE

The SimpliRED® D-dimer assay is a rapid, qualitative test for the detection of cross-linked fibrin degradation products containing the cross-linked D-dimer site in human whole blood. The test can be used by medical professionals at any patient location.

SUMMARY AND EXPLANATION OF THE TEST

During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The fibrin monomers polymerise to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed. Fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, but only degradation products from cross-linked fibrin contain D-dimer⁽¹⁻³⁾. Therefore cross-linked fibrin degradation products (XL-FDP) are a specific marker of fibrinolysis.

TEST PRINCIPLE

SimpliRED® D-dimer is an autologous red cell agglutination assay. The active agent is a chemical conjugate of a monoclonal antibody specific to D-dimer linked to a monoclonal antibody, which binds to the red blood cell surface⁽⁴⁾.

The conjugate will coat the red blood cells but will not cause agglutination in samples with normal levels of XL-FDP (that is levels of XL-FDP below 0.20 mg/L). XL-FDP present in a blood sample at greater than 0.20 mg/L will bind to the conjugate on the red cells causing crosslinking between conjugate groups of adjacent cells which result in visible agglutination. In the absence of D-dimer, the conjugate attached to the red blood cells does not cause agglutination⁽⁴⁻⁶⁾.

REAGENTS

Composition

1. SimpliRED® D-dimer Test Reagent: a solution containing red blood cell anti-XL-FDP antibody conjugate, stabilizers, 5.0 mg/mL BSA and 0.05% sodium azide as preservative.
2. SimpliRED® D-dimer Positive Control: a solution containing purified human D-dimer fragment, stabilizers, 5.0 mg/mL BSA and 0.05% sodium azide as preservative.
3. SimpliRED® D-dimer Negative Control: a 0.9% saline solution containing 0.05% sodium azide as preservative.

Warnings and Precautions

- For *In Vitro* Diagnostic Use Only.
- Reagents contain sodium azide (0.05%). Do not ingest or allow to contact skin or mucous membranes.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sink, flush with a large volume of water to prevent azide build up.
- SimpliRED® D-dimer Positive Control contains components of human origin and this material has been tested by FDA approved methods for antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found to be negative. As complete absence from infectious agents can never be assured, the reagent should be treated as potentially infectious. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2⁽⁷⁾.

Preparation for Use

Reagents require no preparation and are ready for use when brought to room temperature.

Storage And Stability

Store reagents at 2°C to 8°C. Do not freeze.
Do not use reagents past the expiration date stated on the package label.

Indication of Reagent Deterioration

A Positive Control is included in the kit. Failure of this control to cause agglutination is an indication of loss of activity in the kit reagents.

SPECIMEN COLLECTION AND PREPARATION

The SimpliRED® D-dimer test is designed for use with freshly collected capillary or venous whole blood.

Venous blood collected into sodium citrate or heparin as anticoagulant is acceptable. Anticoagulated blood should be tested within 4 hours from the time of specimen collection if kept at 18°C - 24°C⁽⁸⁾, or tested within 24 hours if stored refrigerated. Blood without anticoagulant should be used immediately after collection.

Samples showing evidence of clotting are unsuitable for testing.

PROCEDURE

Materials Provided:

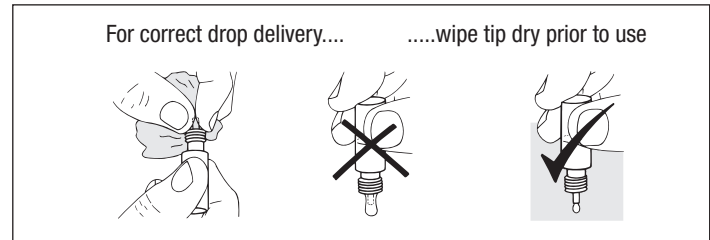
Test Reagent	1 x 1.0 mL (Red Cap)	Sufficient for 40 tests
Negative Control	1 x 1.0 mL (Black Cap)	Sufficient for 40 tests
Positive Control	1 x 0.6 mL (Yellow Cap)	Sufficient for 40 tests
Agglutination Trays	x 40	Two wells each, for agglutination reaction
Stirrers	x 40	White plastic stirrers for mixing
Instructions for Use		

Materials Required But Not Provided:

A timing device suitable for the measurement of the 2 minute reaction time
Pipette and tips capable of dispensing 10 µL
Disposable gloves

Procedural Notes:

- Mix the reagents before use but do not shake.
- Prior to each use, the dropper bottle tips must be wiped dry with a tissue.
- Dropper bottles must be held vertically when dispensing drops of reagent.
- Drop the reagents into the centre of the wells and do not touch dropper tips to any blood sample or other liquid.
- Do not use drops that clearly contain too little or too much reagent.
- Do not touch the inside surface of the reaction wells. Fingerprints may cause uneven wetting.
- Care should be taken not to switch bottle caps. The vial labels and caps have matching colours to assist in identification.
- Treat all patient samples as if they are potentially infectious and observe recommended handling precautions.



A. Test Method:

1. Bring reagents to room temperature.
2. Mix whole blood samples gently but thoroughly and DO NOT ALLOW THE CELLS TO SETTLE OUT.
3. For each test sample, pipette 10 µL of whole blood into each of the reaction wells labelled as “ - ” (Negative Control well) and “ TEST ” (Test well) on an agglutination tray. Use a new tip for each sample. Discard used tips in a biohazard bag.
4. Add one drop of Negative Control (black cap) to the Negative Control well.
5. Add one drop of Test Reagent (red cap) to the Test well.
6. Using a plastic stirrer (provided), mix the contents of the Negative Control well followed by the Test well for approximately 3 to 5 seconds, ensuring thorough mixing and spreading of reagent across the entire well surface. Discard the used stirrer into a biohazard bag.
7. Mix by gentle rocking of agglutination tray for 2 minutes.
8. Examine each well for the presence of agglutination and record the results.
9. The result is positive if the Test well shows any agglutination compared to the Negative Control well. If the Negative Control agglutinates the test is invalid.
10. If the Test result is negative, add one drop of Positive Control (yellow cap) to the Test well and rock the tray. A visible agglutination reaction should be apparent within 15 seconds. The absence of any agglutination renders the test invalid.
11. Discard the used agglutination tray into a biohazard container - do not re-use.

B. Positive and Negative Control QC Method

Follow the directions in the Test Method from steps 1 to 9 (inclusive), but substitute the following procedure for step 3 of the Test Method.

- a. Use a whole blood sample that has a negative D-dimer test result.
- b. Pipette 10 µL of the D-dimer negative whole blood into both the Negative Control well and the Test well of an unused agglutination tray.
- c. Add one drop of SimpliRED® D-dimer Positive Control (yellow cap) to the Test well only.

Quality Control:

A Negative Control and a Positive Control are provided in the kit. The Negative Control should be run with each specimen that is tested and should be used as a comparative reference for absence of agglutination. If agglutination occurs with the Negative Control, the test is invalid. The Positive Control reagent should also be used to confirm the validity of a negative SimpliRED® D-dimer test result (refer to step 10, Test Method). Failure of the Positive Control to cause agglutination is an indication of loss of reactivity of the kit reagents.

The Positive and Negative Control QC Method (B) should be tested at intervals as required by local, state and/or federal regulations or accreditation programs.

Interpretation of Results:

For the correct interpretation of results the agglutination pattern in both wells should be noted.

- **Positive Result**
Visible evidence of agglutination present in the Test well when compared to the Negative Control well.
- **Negative Result**
No agglutination visible in the Test well when compared to the Negative Control well, followed by the formation of a positive agglutination reaction when the confirmatory test is performed with the Positive Control reagent (refer to step 10, Test Method).
- **Results are invalid if**
 - agglutination occurs in the Negative Control well.
 - the Positive Control fails to agglutinate.

Tests with invalid results should be repeated.

LIMITATIONS OF THE PROCEDURE

The SimpliRED® D-dimer assay procedure and interpretation of results must be followed closely. Failure to do so will render the test result invalid. A clinical diagnosis should not be based on the results of SimpliRED® D-dimer alone. Clinical signs and other relevant test information should be included in the diagnostic decision.

Normal hematocrit values are 0.45 ± 0.05 (L/L) for men and 0.41 ± 0.05 (L/L) for women⁽⁹⁾. Hematocrit values outside the range of 0.40-0.50 (L/L) have not been evaluated and may affect the performance of the SimpliRED® D-dimer test.

The presence of cold acting auto-antibodies (cold agglutinins) in patient specimens can cause agglutination of the patient's own red blood cells⁽¹⁰⁾. In the SimpliRED® D-dimer whole blood agglutination assay this may cause agglutination of the Negative Control. This invalidates the test result.

EXPECTED VALUES

Elevated levels of XL-FDP indicate reactive fibrinolysis and are found in a number of clinical conditions including deep vein thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation (DIC) and other coagulation disorders⁽¹¹⁻¹⁵⁾. XL-FDP levels have also been used as a predictive indicator in acute myocardial infarction. Evaluation of XL-FDP levels have been used to monitor thrombolytic therapy with streptokinase and tissue plasminogen activator⁽¹⁶⁻¹⁷⁾. Elevated levels of XL-FDP as an indication of reactive fibrinolysis have also been reported in surgery, trauma, sickle cell disease, liver disease, severe infection/sepsis, inflammation and malignancy⁽¹⁸⁻¹⁹⁾.

SPECIFIC PERFORMANCE CHARACTERISTICS

1. Method Comparison

In a hospital study of 172 whole blood samples⁽⁶⁾, the SimpliRED[®] D-dimer assay was compared to a laboratory based latex test (AGEN DIMERTEST[®] Latex Kit) used for the diagnosis of thrombotic disease. The patient samples consisted of:

- 101 normal (average D-dimer concentration 0.09 mg/L)
- 21 deep vein thrombosis (average D-dimer concentration 0.96 mg/L)
- 18 pulmonary embolism (average D-dimer concentration 0.80 mg/L)
- 32 disseminated intravascular coagulation (average D-dimer concentration 0.96 mg/L)

A comparative analysis yielded the following results:

Normal Group: n =101

SimpliRED [®] D-dimer Result	Latex Test Result	
	(+)	(-)
(+)	0	0
(-)	1	100

Thrombotic Disease Group: n=71

SimpliRED [®] D-dimer Result	Latex Test Result	
	(+)	(-)
(+)	71	0
(-)	0	0

The conclusion of the study was that results of SimpliRED[®] D-dimer are comparable to the laboratory based latex test.

2. Clinical Correlation

An independent, prospective, non-interventional study performed at 6 US teaching hospitals⁽²⁰⁾:

At referral, 380 patients with suspected pulmonary embolism (PE) were tested with SimpliRED[®] D-dimer and alveolar dead-space fraction measurement prior to standard testing for PE. This study used arterial blood for SimpliRED[®] testing.

SimpliRED [®] D-dimer Result	Confirmed PE *	
	(+)	(-)
(+)	60	104
(-)	4	212

*Clinical diagnosis confirmed either by radionuclide lung scan or by contrast enhanced helical chest computed tomography, plus selective use of pulmonary angiography and venous ultrasonography and 6 month follow up.

Summary:

Results of SimpliRED[®] D-dimer testing:

Sensitivity: 93.8 % (95% CI = 84.8 - 98.3) Specificity: 67.1% (95% CI = 61.9 - 72.3)

Pulmonary embolism cannot be safely refuted on the basis of a normal SimpliRED[®] D-dimer assay result alone. SimpliRED[®] D-dimer should be used in conjunction with other clinical data.

3. Specificity

The cross-linked fibrin degradation products D-dimer, D-dimer E, and high molecular weight derivatives are all recognised by the D-dimer specific monoclonal antibody used in the SimpliRED[®] D-dimer whole blood agglutination assay. No binding was found to the fibrinogen degradation products, X, Y, D and E or to fibrinogen to 1000 mg/L⁽⁴⁾.

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ORDERING INFORMATION:

SimpliRED[®] D-dimer[®]
Cat. No. DSRK4

MANUFACTURED BY:

BBInternational
Alchemy House
Tom McDonald Avenue
Medipark, Dundee
DD2 1NH, UK

FOR FURTHER INFORMATION CONTACT:

UNITED KINGDOM:

BBInternational
Telephone: +44 (0) 2920 747232
Facsimile: +44 (0) 2920 747242
Email: simplired@britishbiocell.co.uk
www: <http://www.d-dimer.co.uk>

SYMBOLS KEY

 Consult Instructions for Use

 For In Vitro Diagnostic Use

 Manufactured by

2°C to 8°C Store at 2°C to 8°C

 Batch Code


 "Use By" date

 Catalogue Number

 CE Mark

 Test Reagent

 Positive Control

 Negative Control