An immunoagglutination test for XL-FDP in human plasma.

REF 800DB
For In Vitro Diagnostic Use

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INTENDED USE
Intended for the rapid qualitative or semi-quantitative evaluation of circulating derivatives of cross-linked fibrin degradation products (XL-FDP) in human plasma.

SUMMARY & TEST PRINCIPLE
During blood coagulation, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerize 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, including cross-linked fibrin degradation products (XL-FDP). Degradation products from cross-linked fibrin also contain the D-dimer protein molecule. Therefore, XL-FDP is a specific marker of fibrinolysis. ActiScreen XL-FDP is a rapid immunoagglutination assay utilizing latex beads coupled with a highly specific monoclonal antibody. XL-FDP present in a plasma sample binds to the coated latex beads, which results in visible agglutination occurring when the concentration is above the upper limit of detection for the assay.

REAGENTS
Immunoagglutination Reagent (White Cap): 1 x 2.0 mL dropper bottle containing a 0.83% suspension of latex particles coated with mouse monoclonal anti-XL-FDP antibody, 10 mg/mL BSA and 0.1% sodium azide.
Postive Control (Yellow Cap): 1 x 0.6 mL dropper bottle containing a solution of purified human XL-FDP fragments, 5 mg/mL BSA and 0.1% sodium azide.
Negative Control (Black Cap): 1 x 0.6 mL dropper bottle containing a buffer solution containing 5 mg/mL BSA and 0.1% sodium azide.
Buffer: 1 x 20.0 mL 10 mM phosphate buffer solution with 0.1% sodium azide.

WARNINGS & PRECAUTIONS
For In Vitro Diagnostic Use Only. Harmful if swallowed. Avoid contact with skin and eyes. Wear suitable protective clothing. CAUTION: All reagents in contain sodium azide (0.1%) as preservative. Do not ingest or allow to contact skin or mucous membranes. Do not empty into drains as sodium azide may form explosive azides in metal plumbing. Use proper disposal procedures. CAUTION: The Positive Control contains components of human origin. Each individual blood donation intended for the production of this reagent is tested for HBsAg, anti-HCV, anti-HIV1 and anti-HIV2. Only donations with negative findings are employed. As complete absence of infectious agents can never be assured, all materials derived from human blood should be treated as potentially infectious and handled with due care following the precautions recommended for biohazardous material.

REAGENT PREPARATION & STABILITY
Refer to outer package and vial labels for expiration date. Test kit reagents are stable until the expiration date on the vial label when stored at +2°C - +8°C. DO NOT FREEZE. Reagents require no reconstitution and are ready for use when brought to room temperature (+20°C - +25°C).

INDICATION OF REAGENT DETERIORATION
Reagent deterioration is indicated by failure of the Immunoagglutination Reagent to agglutinate with the Positive Control, agglutination with the Negative Control, or evidence of microbial contamination.

SAMPLE COLLECTION AND PREPARATION
Plasma prepared from whole blood anticoagulated with sodium citrate is recommended. The use of EDTA and heparin will result in an increased level of false positive reactions. After separation of the plasma by centrifugation (1500 x g for 15 minutes at 4°C - 10°C), specimens may be tested directly for the presence of XL-FDP. Defibrination of the plasma is not recommended. Plasma may be stored at -20°C with a stability of 2 weeks. Thaw frozen specimens rapidly at 37°C and centrifuge before testing. Refer to NCCLS publication H21-A3 for further instructions on specimen collection, handling and storage.

PROCEDURE

Materials Provided
- Immunoagglutination Reagent – 1 x 2.0 mL (White Cap)
- Negative Control – 1 x 0.6 mL (Black Cap)
- Positive Control – 1 x 0.6 mL (Yellow Cap)
- Buffer – 1 x 20.0 mL
- Test Cards – 10 x 8 wells for agglutination reaction
- Plastic Stirrers – 1 x 60 for sample mixing
- Instructions for Use

Materials Required But Not Provided
- Precision pipettes and tips (20 µL, 100 µL)
- Plastic test tubes and rack
- Stopwatch or timing device
- Disposable gloves
- Tissue (for wiping dropper bottle tips)

Important! – Equilibrate reagents to room temperature (+20°C - +25°C) before use. Immunoagglutination Reagent should be mixed by inversion, not shaken, immediately prior to use. 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 and/or controls. For correct drop delivery, wipe tip dry prior to use. When dispensing reagent and/or control, hold bottle vertically to ensure proper delivery.

A. Qualitative Method
1. Take one disposable test card. Avoid touching the reading surface. Mark, or make note of, positions on the test card for specimens and, as needed, for positive and negative controls.
2. Hold the Immunoagglutination Reagent dropper bottle vertically and place one drop of the reagent within a well on a test card. AVOID touching the surface of the test card.
3. Accurately pipette 20 µL of undiluted plasma, or one drop of control solution, inside the same well next to the drop of Immunoagglutination Reagent. Mix the Immunoagglutination Reagent and test samples with a stirrer until the latex is uniformly distributed.
4. Rock the test card gently by hand for exactly 3 minutes.
5. At exactly 3 minutes, check for agglutination under a strong light source. (NOTE: If test reading is delayed beyond 3 minutes, the latex suspension may dry out giving a false agglutination pattern. If this is suspected, the specimen must be retested).
6. Discard the Test Card and stirrer into a biohazard container and do not re-use.

B. Semi-Quantitative Method
1. Prepare doubling dilutions of the test plasma with Buffer Solution as follows:
   1:2 dilution: Add 100µL of plasma to 100µL of Buffer.
   1:4 dilution: Add 100µL of 1:2 dilution to 100µL Buffer.
   1:8 dilution: Add 100µL of 1:4 dilution to 100µL Buffer.
2. Repeat this procedure to extend the dilution series as desired. Test each dilution as described in the qualitative method (Procedure A).

QUALITY CONTROL
It is recommended that both Positive and Negative Controls be included in each batch of tests to ensure proper functioning of the system. Control solutions should be tested by the same procedures as patient samples. For the qualitative screening procedure, a positive result (agglutination) can be obtained by substituting the ActiScreen XL-FDP Positive Control for the plasma in steps 3-6. Conversely a negative result (no agglutination) can be obtained by substituting ActiScreen XL-FDP Negative Control for the plasma in steps 3-6. When performing the semiquantitative procedure, it is recommended that testing of serial dilutions of the Positive Control be conducted. ActiScreen XL-FDP Latex Positive Control consists of a solution of human XL-FDP at a level of approximately 800 nM/L (0.80 mg/L). Failure to obtain expected results with either positive or negative controls may be indicative of reagent and/or control contamination.

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RESULTS

A. Qualitative Method
For the qualitative method protocol, the following pattern of results should be obtained.

<table>
<thead>
<tr>
<th>UNDILUTED PLASMA</th>
<th>XL-FDP CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Less than 200 ng/mL (0.20 mg/L)</td>
</tr>
<tr>
<td>Positive</td>
<td>Greater than 200 ng/mL (0.20 mg/L)</td>
</tr>
</tbody>
</table>

Note: All values in ng/mL and mg/L are approximate.

B. Semiquantitative Method
Approximate levels of XL-FDP for specimen dilutions are shown in the table below. As with all semiquantitative tests, some variability in dose-response can be expected.

<table>
<thead>
<tr>
<th>~ XL-FDP Levels ng/mL (mg/L)</th>
<th>SAMPLE DILUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200 (&lt;0.20)</td>
<td>-</td>
</tr>
<tr>
<td>200 – 400 (0.20 – 0.40)</td>
<td>±</td>
</tr>
<tr>
<td>400 – 800 (0.40 – 0.80)</td>
<td>±</td>
</tr>
<tr>
<td>800 – 1600 (0.80 – 1.60)</td>
<td>+</td>
</tr>
<tr>
<td>1600 – 3200 (1.60 – 3.20)*</td>
<td>+</td>
</tr>
</tbody>
</table>

* = agglutination, ** = no agglutination
* Levels of XL-FDP greater than 3200 ng/mL (3.20 mg/L) can be estimated by further dilutions beyond 1:8.

LIMITATIONS OF THE PROCEDURE
In a study of samples from patients with rheumatoid arthritis, 17 were found to agglutinate with ActiScreen XL-FDP. In all 17 samples, the agglutination could be inhibited by the addition of the XL-FDP specific monoclonal antibody DD3B6/22, but not with a non-specific monoclonal antibody of the same subgroup, IgGκ. This suggests that ActiScreen XL-FDP is insensitive to rheumatoid factor disturbances. In addition, ActiScreen XL-FDP does not cross-react with fibrinogen, factor XIIIa cross-linked fibrinogen13, or FDP4.

No assay interference was demonstrated with ActiScreen XL-FP Latex with specimens containing potential interferents at the following concentrations:

- Bilirubin: 0.2 mg/mL
- Hemoglobin: 5.0 mg/mL
- Lipids (triglycerides): 30 mg/mL
- Protein (globulin): 0.06 g/mL

As with any laboratory test, detection of elevated levels of XL-FDP in a sample should be used in conjunction with other clinical information in forming a diagnosis. Use of this kit has been validated for the presence of XL-FDP levels in patients suspected of having DIC. This kit has been validated for the presence of XL-FDP levels in patients suspected of having DIC.

EXPECTED VALUES
Clinical diagnosis should not be based on the results of ActiScreen XL-FDP alone. Clinical signs and other relevant test information should be included in the diagnostic decision. A positive result, indicating active fibrinolysis, should be obtained with ActiScreen XL-FDP when XL-FDP levels are at, or greater than, approximately 200 ng/mL (0.20 mg/L). Plasma specimens from normal subjects are expected to give negative results because their plasma XL-FDP concentrations are typically less than 200 ng/mL (0.20 mg/L). Due to many variables that may affect results, each laboratory should establish its own normal range. Elevated levels of XL-FDP (containing the D-dimer domain) have been demonstrated in patients by a combination of immunoprecipitation and gel electrophoresis techniques13,14. Monoclonal antibody based D-dimer assays allow the specific detection of the D-dimer domain and are of diagnostic value in disseminated intravascular coagulation (DIC) and acute vascular diseases, including pulmonary embolism (PE) and deep venous thrombosis (DVT), conditions that are difficult to detect reliably by clinical examination15.

The amount of XL-FDP detected in a specimen will depend on several interrelated factors in vivo, such as the severity of the thrombotic episode, the rate of cross linked fibrin formation, and the time elapsed after the thrombotic event until blood is drawn from the patient. 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 malignancy16. Levels may also rise during normal pregnancy, but very high levels are associated with complications17.

PERFORMANCE CHARACTERISTICS
Reproducibility – Intra-assay (within-run) reproducibility was determined for 10 replicates of 3 plasma samples that contained different levels of XL-FDP. The results were equivalent for all replicates. Inter-assay (run-to-run) reproducibility was determined using 10 plasma samples with XL-FDP titers ranging from 1 to 16. In 10 runs, the replicates of these specimens did not vary by more than one titer.

Specificity – Plasma from 170 apparently healthy, voluntary blood donors was tested using ActiScreen XL-FDP. A negative result was obtained for 162 of the samples, equating to a specificity of 95.3%.

Method Comparison – ActiScreen XL-FDP and another commercially available agglutination procedure, as the reference method, tested 145 plasma samples from patients judged to be suffering from, or having a high probability for thrombotic episode. The correlation coefficient was r = 0.94 and the regression equation was y = 1.19x. In an anticoagulant study of 50 parallel citrated, EDTA and heparin plasma samples, the correlation between the titers obtained with ActiScreen XL-FDP and the expected titers (based on ELISA XL-FDP values) was r = 0.91 for citrated samples, r = 0.73 for EDTA samples and r = 0.78 for heparin samples. Citrate is the anticoagulant of choice.

REFERENCES