Investigation of Thrombophilia

ACT Pathology offers a range of tests for the investigation of thrombophilia. These include Protein C, Protein S, antithrombin (AT), activated protein C resistance (APCR) and molecular tests for the detection of factor V Leiden mutation and prothrombin gene mutations. We also offer a comprehensive panel of tests for antiphospholipid antibody syndrome antibodies to cardiolipin and beta2 glycoprotein 1 (IgM & IgG), and lupus anticoagulant. Homocysteine assay is also available as a test for thrombophilia.

Patients should be considered for all of the above tests if they are less than 50 years old with recurrent thrombosis, or if they have had a single thrombotic event and have a positive family history. Current recommendations characterise these patients as “strongly” thrombophilic. Other clinical features that may suggest an inherited thrombotic disorder include thrombosis at unusual sites, heparin resistance and warfarin induced skin necrosis.

The most common findings associated with venous thrombosis are Factor V Leiden and Prothrombin gene mutation. Protein C, Protein S and Antithrombin deficiency are far less common and are unlikely to be of relevance in “weakly” thrombophilic patients in whom the initial thrombotic event has occurred after the age of 50 years, particularly in the absence of a family history of thrombosis. Therefore, in these patients, factor V leiden, prothrombin gene mutation and tests for APA are appropriate.

The relevance of the results of thrombophilia screening may be of uncertain significance in some cases, particularly with regard to the Factor V Leiden and Prothrombin mutations, which tend to occur in 5% and 1-4% of the general Caucasian population respectively. Recent studies suggest that the most important predictive factors in terms of recurrence of thrombosis are clinical features and persistent ultrasound abnormalities, rather than the tests for thrombophilia. Also, in most cases, detection of a thrombophilic condition does not alter management, particularly where a clear precipitant for thrombosis is responsible for the thrombotic event.

Tests requested for the investigation of thrombophilia must be individually listed, as “Thrombophilia screen” is not accepted as a valid test or test grouping under H.I.C. rules as applied at ACT Pathology. The Pathology Services Arrangements under Medicare as listed in the Medicare Benefits Schedule book 2005 states that a Pathology service can recover costs for outpatient or private inpatients only where “the request for the test(s) specifically identifies that the patient has a history of venous thromboembolism”, OR “in a first degree relative of a person who has a proven defect” of any one of the following; AT, protein C, Protein S, or APCR
**Factor V Leiden and Prothrombin Gene Mutation Testing**

The Factor V Leiden mutation has been identified as a major cause of familial venous thrombosis and is inherited in an autosomal dominant fashion. Heterozygosity is associated with an 8-fold increased risk of venous thrombosis and homozygosity with an 80-100-fold increased risk.

The prothrombin mutation (G20210A) is linked to increased prothrombin levels. It is inherited independently from the Leiden mutation in an autosomal dominant fashion and is associated with an approximate 3-fold increased risk of venous thrombosis. The incidence of this mutation in the general Caucasian population is approximately 2%.

**Antiphospholipid Antibodies**

Antiphospholipid antibodies are a family of autoantibodies that recognise various phospholipids and/or phospholipid-binding proteins. This antibody family includes lupus anticoagulants, anticardiolipin antibodies and anti-beta2-glycoprotein I antibodies. All confer an increased risk of thromboembolic disease and placental insufficiency.

The diagnosis of Antiphospholipid syndrome, which can occur in isolation, or in association with other systemic autoimmune disease, such as lupus, is made using clinical and laboratory criteria.

**Criteria for the classification of the antiphospholipid syndrome:**

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>Laboratory criteria</th>
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<tr>
<td>1. Thrombosis - arterial, venous or small vessel</td>
<td>Persistently detected (on 2 or more occasions, at least 12 weeks apart) any one of:</td>
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<tr>
<td>2. Complications of pregnancy - recurrent miscarriage</td>
<td>positive aPL, positive Lupus anticoagulant test, moderate to high titre anticardiolipin</td>
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<td>antibodies or abnormal levels of B2 glycoprotein 1 antibodies.</td>
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*To make a definite diagnosis of antiphospholipid syndrome, patients must meet at least one of the clinical AND one of the laboratory criteria.*

**1. Lupus Anticoagulants**

Lupus Anticoagulants (LAC) are often detected during a Full Coagulation Profile (FCP) because they can prolong the APTT and/or PT. (They are named for this *in vitro* effect whereas they have a procoagulant effect *in vivo*). A mixing test, initiated by the laboratory that does not correct the APTT and/or PT indicates either the presence of a LAC, or less commonly an inhibitor of any of the coagulation factors. Tests to order for a Lupus anticoagulant investigation include Full Coagulation Profile (FCP) and Lupus Anticoagulant.
2. Anticardiolipin Antibodies
Anticardiolipin antibodies are measured by enzyme-linked immunoassay. The laboratory tests routinely for IgG cardiolipin antibodies, IgM antibodies may also be requested.

3. Anti-beta2-glycoprotein I Antibodies (B2GP1)
Anti-beta2-glycoprotein I antibodies are measured by enzyme linked immunoassay. The laboratory tests routinely for IgG antibodies, IgM antibodies may also be requested.

Activated Protein C Resistance (APCR)
The APCR test is a sensitive screening test for Factor V mutations, which may lead to an increased risk of thrombosis. APCR is the most common known hereditary predisposition to venous thrombosis. Activated protein C normally degrades activated factors V and VIII by proteolytic change to inhibit coagulation. Individuals with APCR have a mutated Factor V, which is resistant to degradation by activated Protein C. More than 95% of cases are due to a point mutation known as the Factor V Leiden mutation, which is present in the heterozygous form in 3-5% of the general Caucasian population and is less common in other ethnic groups. APCR has been found in 20% of patients with first episode thrombosis and in 50% of patients with familial thrombosis.

Protein C
Protein C deficiency occurs in 0.14% to 0.5% of the population. Protein C deficiency has been found in 3% of patients with a first venous thrombosis and no known malignancy. Heterozygous protein C deficiency increases the risk for venous thrombosis sevenfold. It is more likely to be of relevance in young (< 50 years) patients with a positive family history of thrombosis.

Protein S
Protein S is a cofactor for activated Protein C mediated degradation of the coagulation factors Va and VIIIa. Hereditary protein S deficiency occurs in 0.1% of the population. It has been found in up to 7% of patients with thrombosis, particularly those occurring in young (<50 years) patients with a positive family history of thrombosis.

Antithrombin
Antithrombin is a powerful physiological coagulation inhibitor, which inhibits the activity of thrombin and factor Xa and to a lesser degree the activity of factors IXa, XIa and XIIa and Kallikrein. Antithrombin deficiency occurs in 0.17% of the population and has been found in 1.1% of patients with venous thrombo-embolism. Some reports suggest that antithrombin deficiency increases the risk for venous thrombosis fivefold.

Specimen type and test availability
Protein C, Protein S, AT III, Lupus Anticoagulant, and APC Resistance.
Container type: 2 x 2.7ml Citrate (Blue Top) filled to the line.
Availability: Tests are run weekly in the Haematology laboratory.

Homocysteine, Anticardiolipin (ACA), B2 glycoprotein I (B2GPI)
Container type: 1 x Serum (Red top) is enough for all 3 tests.
Availability: Tests are run weekly in Immunology lab.

Factor Va Leiden & prothrombin gene mutation
Container Type: 1 x 4 ml (pink topped) or 10 ml (purple topped) EDTA.
Availability: Tests are run weekly in Molecular laboratory.

Full Coagulation Profile (FCP)
Container Type: 1 x 2.7ml Citrate (Blue Top) filled to line
Availability: 24 hours

References:

5. Medicare Benefit Schedule (MBS) book 2005; pg 580