### GUIDELINES FOR THROMBOPHILIA TESTING

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Purpose of Issue/Description of Change</th>
<th>Review Date</th>
</tr>
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<tr>
<td>1.0</td>
<td>April 2010</td>
<td>New guideline</td>
<td>April 2013</td>
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**Status**: Final

**Publication Scheme**: No listing

**FOI Classification**: Release without reference to author

**Function/Activity**: Clinical and Haematology Laboratory

**Record Type**: Guideline

**Project Name**: N/A

**Key Words**: Thrombophilia

**Standard**: BCSH guidelines

**Scope / Location**: Trust wide and Haematology Laboratory

**Author**: Dr Emma Harris  
**Date/s**: March 2010

**Approval and/or Ratification Body**: Hospital Thrombosis Committee  
**Date**: March 2010
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1. INTRODUCTION

1.1. Purpose

The term “thrombophilia” means an inherited or acquired predisposition to venous thromboembolism (VTE) although in the UK it is usually thought of in the context of inherited abnormalities diagnosed on laboratory testing. Whilst it is natural for both patient and clinician to want to look for the cause of a thrombosis, it is generally recommended only to perform thrombophilia testing where the management of the patient will be altered by the results.

It is important to realise the limitations of thrombophilia testing. Unless testing for a specific heritable thrombophilic defect in a family known to carry that defect, a negative result does not exclude the possibility of an inherited predisposition to thrombosis. Clearly new thrombophilic defects will continue to be discovered. This is particularly the case in patients with a strong family history of thrombosis. The patient’s personal and family history of thrombosis are almost always more important than the thrombophilia test result and a negative result should not be allowed to provide false reassurance for the patient and clinician. It is now apparent that testing for heritable thrombophilia does not usually predict likelihood of recurrence in unselected VTE patients and does not reduce recurrence rates. There is a low risk of thrombosis in affected asymptomatic relatives and the results of thrombophilia tests are frequently misinterpreted.

A review of thrombophilia screening at HDFT in 2006-2007 identified that around £24,500 was spent annually on thrombophilia screening, of which approximately 50% was inappropriate. This guideline, along with changes to the ICE requesting and laboratory booking-in system, aims to reduce the number of inappropriate tests performed.

1.2. Scope

This guideline is aimed at anyone requesting thrombophilia testing, both in the Trust and in GP practices.

2. SUPPORTING INFORMATION

2.1. Venous thrombosis

Diagnosing a heritable thrombophilia will rarely alter a patient’s management. In fact, whilst the presence of a heritable thrombophilic defect increases the risk of first thrombosis to varying degrees, it does not strongly predict the risk of recurrence after discontinuation of anticoagulant treatment, in unselected VTE patients.
Guidelines for Thrombophilia Testing

It used to be thought that a potential advantage of testing would be to identify affected but asymptomatic family members in order to try to prevent a first thrombosis by addressing periods of high risk or lifestyle modification. However, individual risk is affected by multiple genetic and environmental factors which will be different even amongst first degree relatives. Case finding of asymptomatic relatives of patients with VTE and thrombophilia has not been shown to reduce the incidence of VTE and the annual risk of unprovoked thrombosis in affected family members is low. If a family history suggests a high degree of genetic penetrance then it might be reasonable to test a symptomatic patient and then their relatives, with a view to enhanced prophylaxis at times of high risk in affected members.

The utility of testing in patients with upper limb thrombosis, cerebral sinus thrombosis and intra-abdominal vein thrombosis is unknown. These patients should be discussed with a Consultant Haematologist prior to testing. Testing is NOT indicated in patients with retinal vein occlusion.

2.1.1. Combined Oral Contraceptive Pill / Hormone Replacement Therapy

Any woman wishing to take the Combined Oral Contraceptive Pill (COCP) or Hormone Replacement Therapy (HRT) who has a family history of VTE in a first degree relative should consider an alternative contraception or transdermal HRT.

If the affected relative has not had thrombophilia testing, or they have had a negative result, testing in the asymptomatic patient will provide an uncertain estimate of risk and is not recommended.

If a symptomatic first degree relative has been tested and has a heritable thrombophilia, alternatives should still be considered first in the patient as a negative test does not exclude an increased risk of VTE. Testing for heritable thrombophilia may assist counselling of selected women, particularly if a high risk thrombophilia has been identified in the symptomatic relative.

2.2. Arterial thrombosis

Arterial thrombosis is a multi-factorial condition, of which most risk factors don’t overlap with those for venous thrombosis. No firmly established increased risk for arterial cardiovascular diseases by heritable thrombophilia exists. Treatment and secondary prevention should be in relation to established cardiovascular risk facts. Testing for heritable thrombophilia is NOT recommended.

However anti-phospholipid syndrome may present with arterial thrombosis and should be tested for if this occurs at a young age, particularly in the absence of other risk factors.
2.3. Pregnancy complications

There is evidence of an association between heritable thrombophilia and pregnancy loss including early and late pregnancy loss, pre-eclampsia and intra-uterine growth restriction. However the role of anti-thrombotics in subsequent pregnancies has not been established and therapeutic decisions should be made on clinical circumstances and not the results of thrombophilia testing. Randomised controlled trials to address this question are in progress. This contrasts with anti-phospholipid syndrome in which a causal link to pregnancy failure is more firmly established.

2.4. The thrombophilia screen

Components of a full thrombophilia screen are:

- **Antithrombin** is a major inhibitor of blood coagulation and is essential for effective heparin therapy. It inhibits the coagulation proteases including IIa, IXa, Xa and XIa. **Antithrombin deficiency** is very rare (prevalence 0.02%) but has a high risk of venous thrombosis (5-10x relative risk (RR) for first VTE).

- **Protein C** is a vitamin K-dependent protein made by the liver. It is a natural anticoagulant. It is converted to activated protein C (APC) by thrombin. APC inactivates factors Va and VIIIa. **Protein C deficiency** is rare (prevalence 0.2%). There is variable increased thrombotic risk (4-6x RR for first VTE).

- **Protein S** is a vitamin K-dependent protein made by the liver. It is the co-factor for the anticoagulant activity of APC. It circulates in a free form (40%) or bound to the acute phase C4b-binding protein (60%). Only the free form is functional and only this is measured in the thrombophilia screen. **Protein S deficiency** is rare (prevalence 0.03-0.13%) and is associated with a variable increased thrombotic risk (1-10x RR for first VTE).

  - For all three natural anticoagulant factors, environmental factors may lead to acquired deficiency.
  - Severe liver disease reduces antithrombin, protein C and protein S.
  - Severe vitamin K deficiency, usually due to warfarin therapy, reduces protein C and protein S.
  - Pregnancy and oestrogen therapy both reduce protein S. Protein S falls very early in pregnancy and low protein S can persist for 6-8 weeks even after early miscarriage.

- **Factor V Leiden mutation and APC Resistance Assay (APCR)** If a patient’s plasma does not produce the appropriate anticoagulant response to APC, this is termed **APC Resistance**. The most common cause for this is the **Factor V Leiden mutation** (FVL) which produces a factor V molecule that is resistant to cleavage by APC. FVL is identified using PCR technology. PCR testing is carried out on all samples that have a reduced APCR or have a family history of FVL. FVL is the most prevalent thrombotic risk factor known in the Caucasian population (around 5%). Heterozygotes have a modest increase in
the risk of thrombosis (3-5x RR for first VTE). Homozygotes are much less common but have a much higher thrombotic risk (80x RR).

- APC Resistance may also be caused by other rare mutations and by environmental factors such as pregnancy, combined oral contraceptive pill and hormone replacement therapy.

- **Prothrombin Gene Mutation (G-20210-A)** causes elevated levels of prothrombin in the circulation. Prothrombin is the precursor of thrombin which is a key enzyme in blood coagulation. This mutation is tested for by PCR. Heterozygotes are common in the Caucasian population (around 3%) and have a small increased risk of thrombosis (2-3x RR for first VTE). Homozygotes or compound heterozygotes with FVL have a significantly greater risk of thrombosis.

- **Lupus Anticoagulant and Anti-Cardiolipin antibodies** are carried out to look for Anti-Phospholipid Syndrome (APS). This is an acquired thrombophilic state. APS is an autoimmune disease characterised by thrombosis or pregnancy complications in the presence of anti-phospholipid antibodies. Clinical criteria include
  - Venous or arterial thrombosis
  - One or more unexplained fetal deaths after 12 weeks of gestation
  - Three or more unexplained consecutive miscarriages before 12 weeks of gestation

  Laboratory criteria include
  - Lupus anticoagulant (LA) present in the plasma
  - Moderate or high titres of anti-cardiolipin antibody (ACLA)
  - Performed twice, 12 weeks apart, positive on both occasions

- LA results in the prolongation of coagulation tests dependent on phospholipids eg APTT or DRVVT (dilute Russell’s viper venom test), without specifically inhibiting any individual coagulation factor. It is associated with a range of autoimmune disorders, infections or drugs.

- ACLA are performed as part of the thrombophilia screen but are tested separately in the immunology laboratory at Leeds General Infirmary.

### 2.5. Consent and the Human Tissue Act

Under the terms of the Human Tissue Act (2004), obtaining scientific or medical information about a living or deceased person which may be relevant to another person (including a future person) is deemed a ‘scheduled purpose’. This means that diagnostic testing where genetic information is obtained, such as in a thrombophilia screen, requires specific consent. A ‘Consent for Genetic Investigation’ form has been developed to address this issue (see Appendix 2). It is a Trust requirement that doctors within HDFT use this form for informed consent.

The Human Tissue Authority accepts that the manner in which consent is obtained and recorded will depend on circumstances; this may be different for the GP setting compared with the Hospital setting. Written consent is not a requirement of the Act but good documentation is. GPs may wish to use the
Trust consent form or others that are widely available eg http://clingensoc.org/Docs/Consent_conf_v2.pdf

In October 2001, the Association of British Insurers (ABI), in agreement with the Government, announced a moratorium on access by ABI member companies to applicants’ predictive genetic test results. The genetic tests which form part of thrombophilia screen (Factor V Leiden and Prothrombin Gene Mutation) do not need to be declared when applying for life insurance.

3. POLICY

From April 2010, thrombophilia testing will only be carried out on patients who fulfil the following clinical criteria. Requesting should be carried out on ICE. There will be appropriate prompts and a link to these guidelines. Any samples from patients not fulfilling these criteria will not be sent for testing. Whenever possible, the Haematology Laboratory staff will try to contact the requesting clinician before discarding the sample. Any case may be discussed with a Consultant Haematologist, preferably prior to taking the blood samples. Difficult cases may be referred to Dr Emma Harris’ Thursday Haemostasis and Thrombosis Clinic.

3.1. Who should have thrombophilia testing?

Thrombophilia screening is expensive and time-consuming. As already discussed, testing is often performed without a clear idea of what to do with the results. Positive results often cause unjustified concern to the individual. Conversely, a negative test may provide false reassurance. Therefore it is important that screening is targeted at the right people and the requesting clinician should have a plan of how the results will affect management.

Personal history of VTE

- First episode of venous thrombosis (unprovoked or provoked by minor risk factor eg travel) under the age of 50 years
- Oestrogen-provoked venous thrombosis under age of 50 years
- Venous thrombosis at unusual site eg cerebral, mesenteric – discuss with Consultant Haematologist

Full thrombophilia screen

Patients developing warfarin skin necrosis

Protein C and protein S only

Patient with NO personal history of thrombosis but with thrombosis-prone family history of a specific thrombophilic defect

Test only for known defect
NB Case finding of asymptomatic relatives with Factor V Leiden or Prothrombin gene mutation is NOT indicated except in specific circumstances (see Section 2.1.1 on COCP/HRT and later in this section on testing in pregnancy)

Unexplained arterial thrombosis under 60 years

Lupus Anticoagulant / Anti-cardiolipin Antibodies

Pregnancy morbidity

- One or more morphologically normal fetal deaths after 12 weeks of gestation
- Three or more unexplained consecutive miscarriages before 12 weeks of gestation
- One or more preterm births before 34th week due to severe pre-eclampsia, eclampsia or placental insufficiency

Full thrombophilia screen (Testing should be deferred until at least 8 weeks after most recent miscarriage/birth)

For decisions regarding Thromboprophylaxis in pregnancy:

- Women with a previous non-oestrogen related VTE due to a minor provoking factor

- Asymptomatic women without clinical risks sufficient to warrant thromboprophylaxis but with a family history of VTE in first degree relative (thrombophilic status unknown), if the VTE was unprovoked, provoked by a minor risk factor, related to pregnancy or combined oral contraceptive

Full thrombophilia screen

- Asymptomatic women without clinical risks sufficient to warrant thromboprophylaxis but with a family history of VTE in first degree relative (with known thrombophilia), if the VTE was unprovoked, provoked by a minor risk factor, related to pregnancy or combined oral contraceptive

Test only for known defect

Children

Children have a very low thrombotic risk and should not normally be tested. Any possible need for thrombophilia screening in a child should first be discussed with a Consultant Haematologist or Paediatrician.
3.2. **Timing of the test**

- Testing should not be carried out during the acute presentation with thrombosis as the thrombotic process itself affects the results and does not influence management.

- Testing should be delayed until 4-6 weeks after stopping anticoagulant therapy. If the patient is on lifelong warfarin, the laboratory should be informed and a more limited number of tests will be performed.

- Ideally, testing should not be performed when taking the pill or during pregnancy, or for 8 weeks afterwards. Testing may occasionally be undertaken in pregnancy but should only be undertaken in line with RCOG guidelines, by a clinician with understanding of how to interpret the results and how they will affect management.

- Unfractionated heparin interferes with the coagulation based assays and can cause reduced anti-thrombin. Patients on low molecular weight heparin may be tested if the results of the coagulation screen are normal.

3.3. **Sample requirements**

**Full thrombophilia screen**

- 2 x 3.2ml citrate (blue-capped) tube (must be filled to correct level)
- 5mls EDTA (purple-capped)
- 1 x clotted tube (gold-capped)

**For individual tests**

- Antithrombin: 1 citrate (blue-capped) tube
- Protein C: 1 citrate (blue-capped) tube
- Protein S: 1 citrate (blue-capped) tube
- FVL: 1 EDTA (purple-capped) tube
- PGM: 1 EDTA (purple-capped) tube
- LA/ACLA: 2 x citrate (blue-capped) tube and 1 x clotted tube (gold-capped)

**NB:** Samples should be taken in the morning of a Monday, Tuesday or Wednesday in order to allow for transportation to SJUH in good time.
3.4. Reference ranges

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td>Antithrombin</td>
<td>0.8-1.2 IU/ml</td>
</tr>
<tr>
<td>Protein C</td>
<td>0.7-1.4 IU/ml</td>
</tr>
<tr>
<td>Protein S</td>
<td>0.7-1.0 IU/ml</td>
</tr>
<tr>
<td>APC Resistance ratio</td>
<td>Ratio &gt;2.5</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>Reported as</td>
</tr>
<tr>
<td></td>
<td>• normal,</td>
</tr>
<tr>
<td></td>
<td>• abnormal (heterozygote)</td>
</tr>
<tr>
<td></td>
<td>• abnormal (homozygote)</td>
</tr>
<tr>
<td>Prothrombin gene mutation</td>
<td>Reported as</td>
</tr>
<tr>
<td></td>
<td>• normal,</td>
</tr>
<tr>
<td></td>
<td>• abnormal (heterozygote)</td>
</tr>
<tr>
<td></td>
<td>• abnormal (homozygote)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Reported in an interpretive manner</td>
</tr>
<tr>
<td>Anticardiolipin antibodies</td>
<td>0 -17 u/ml</td>
</tr>
</tbody>
</table>

4. ROLES AND RESPONSIBILITIES

Thrombophilia screens should only be requested by clinical staff with sufficient knowledge to interpret the results and understand the clinical significance for the patient. The clinician requesting the test must take informed consent. The laboratory staff should query all requests that do not comply with the policy and get the involvement of a Consultant Haematologist if necessary.

5. CONSULTATION, APPROVAL AND RATIFICATION PROCESS

5.1. Consultation Process

See Appendix 1.

5.2. Ratification Process

The guideline has been ratified at the Hospital Thrombosis Committee.

6. DOCUMENT CONTROL

The guideline will be published in the Trust electronic document library, including the shared area with GPs.

7. DISSEMINATION

The implementation of the guideline will be announced on the intranet homepage. It will also be taken to the Haematology Laboratory staff meeting.
and to the Medical CBU Governance meeting. GPs will be informed in the Pathology Newsletter.

8. REFERENCE DOCUMENTS

Clinical guidelines for testing for heritable thrombophilia, British Committee for Standards in Haematology (in press)
Investigation and Treatment of Couples with Recurrent Miscarriage. RCOG Green Top Guideline Number 17, May 2003
Reducing the Risk of Thrombosis and Embolism during Pregnancy and the Puerperium, RCOG Green Top Guideline Number 37, November 2009

9. APPENDIX 1

| Those listed opposite have been consulted and comments/actions incorporated as required. |
| List Groups and or Individuals Consulted |
| ---------------------------------------- | --------------------------------- |
| Dr Claire Hall, Consultant Haematologist |
| Dr Geraldine Bynoe, Consultant Haematologist |
| Andrew Jackson, Haematology Laboratory Manager |
| Dr Kat Johnson, Consultant Obstetrician |
| Hospital Thrombosis Committee |
| Dr Nigel Weightman, HDFT Designated Individual for Human Tissue Act |
10. APPENDIX 2

Consent for Genetic Investigation

Patient details (or pre-printed label)

Affix label here

Responsible health professional

Job title

Special requirements
(eg other language/other communication method)

To be retained in patient’s notes
I Consent to have a DNA/chromosome Test for myself/my relative (name of relative) because of a family history of, or implications to my family of, ................................................................. (condition)

I confirm that:

<table>
<thead>
<tr>
<th></th>
<th>YES/NO/Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have received information about the condition and its</td>
<td></td>
</tr>
<tr>
<td>implications for myself and my family from</td>
<td></td>
</tr>
<tr>
<td>.........................Doctor/Counsellor.</td>
<td></td>
</tr>
<tr>
<td>I understand that the results of the test may have</td>
<td></td>
</tr>
<tr>
<td>implications for my health, the health of members of my family</td>
<td></td>
</tr>
<tr>
<td>or their children.</td>
<td></td>
</tr>
<tr>
<td>I understand that a positive test result may have</td>
<td></td>
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<tr>
<td>implications for insurances for myself or my family.</td>
<td></td>
</tr>
<tr>
<td>I understand that the test may take some time but that</td>
<td></td>
</tr>
<tr>
<td>I will be contacted again by a Doctor/ Counsellor if the test</td>
<td></td>
</tr>
<tr>
<td>has not been carried out within two years.</td>
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</tr>
<tr>
<td>I understand that I can choose not to proceed with the test at</td>
<td></td>
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<tr>
<td>any time.</td>
<td></td>
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<tr>
<td>I agree that any residual material from my sample can</td>
<td></td>
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<tr>
<td>be stored following analysis for comparison with tests from</td>
<td></td>
</tr>
<tr>
<td>other family members if necessary, and for subsequent</td>
<td></td>
</tr>
<tr>
<td>ethically approved anonymised medical research, education,</td>
<td></td>
</tr>
<tr>
<td>training, clinical audit, performance assessment, public health</td>
<td></td>
</tr>
<tr>
<td>monitoring or quality assurance.</td>
<td></td>
</tr>
<tr>
<td>I wish to know the results of the test.</td>
<td>YES/NO/Not applicable</td>
</tr>
</tbody>
</table>

I understand that the Doctor/Counsellor will not disclose any information about me to a third party without my consent.

Signature of patient/next of kin: .................................................................

I confirm that I have explained the purpose and nature of the test and its implications.

Signature of Doctor/Counsellor: .................................................................

Date:..........................................

Top copy accepted by patient: yes/no (please ring)