HAEMOSTASIS & THROMBOSIS LABORATORY USER GUIDE

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1 INTRODUCTION

This User Guide has been produced to assist both internal hospital and external community users of the Haemostasis and Thrombosis (Haemophilia) Laboratory at Kent and Canterbury Hospital, providing a quick and easy reference guide to the services available. Information is provided on access to the service, operating hours, key contacts for both laboratory and clinical staff, the tests available, specimen requirements, expected turnaround times, the interpretation of results and factors which may affect the results. If any other information is required, please contact the Laboratory directly using the contact details listed in Section 4 below, and we will be very happy to help. All feedback is welcome and will help us develop the User Guide and our services.

Haemostasis is the human body's response to blood vessel injury and bleeding. It involves a coordinated effort between blood vessels, platelets, numerous blood clotting proteins, inhibitors and the fibrinolytic system. A deficiency or exaggeration of any one of these components may lead to either bleeding or thrombosis.

The Haemostasis and Thrombosis Laboratory processes both Haemophilia (bleeding) and Thrombophilia (thrombosis) requests and this user guide will cover both of these aspects of haemostasis.

The Haemostasis and Thrombosis Laboratory is located within the Haemophilia and Thrombosis Centre based at Kent and Canterbury Hospital. The Centre is one of twenty-eight Comprehensive Care Centres nationally for the diagnosis and treatment of haemostasis disorders.

The Laboratory provides the specialist coagulation services for the whole of East Kent Hospitals University NHS Foundation Trust (EKHUFT) and also for some other hospitals in the Kent and Medway region. It offers a full range of specialist investigations for patients with inherited and acquired disorders of haemostasis and thrombosis using state of the art Stago STA-R automated coagulation analysers, other specialist analysers and manual techniques where required.

A 24/7 routine coagulation screening service is provided by the Blood Science Laboratories on all three acute sites across the Trust using the same state of the art Stago STA-R analysers. The Haemostasis and Thrombosis Laboratory provides scientific, technical and training support for the routine coagulation service.

2 LOCATION

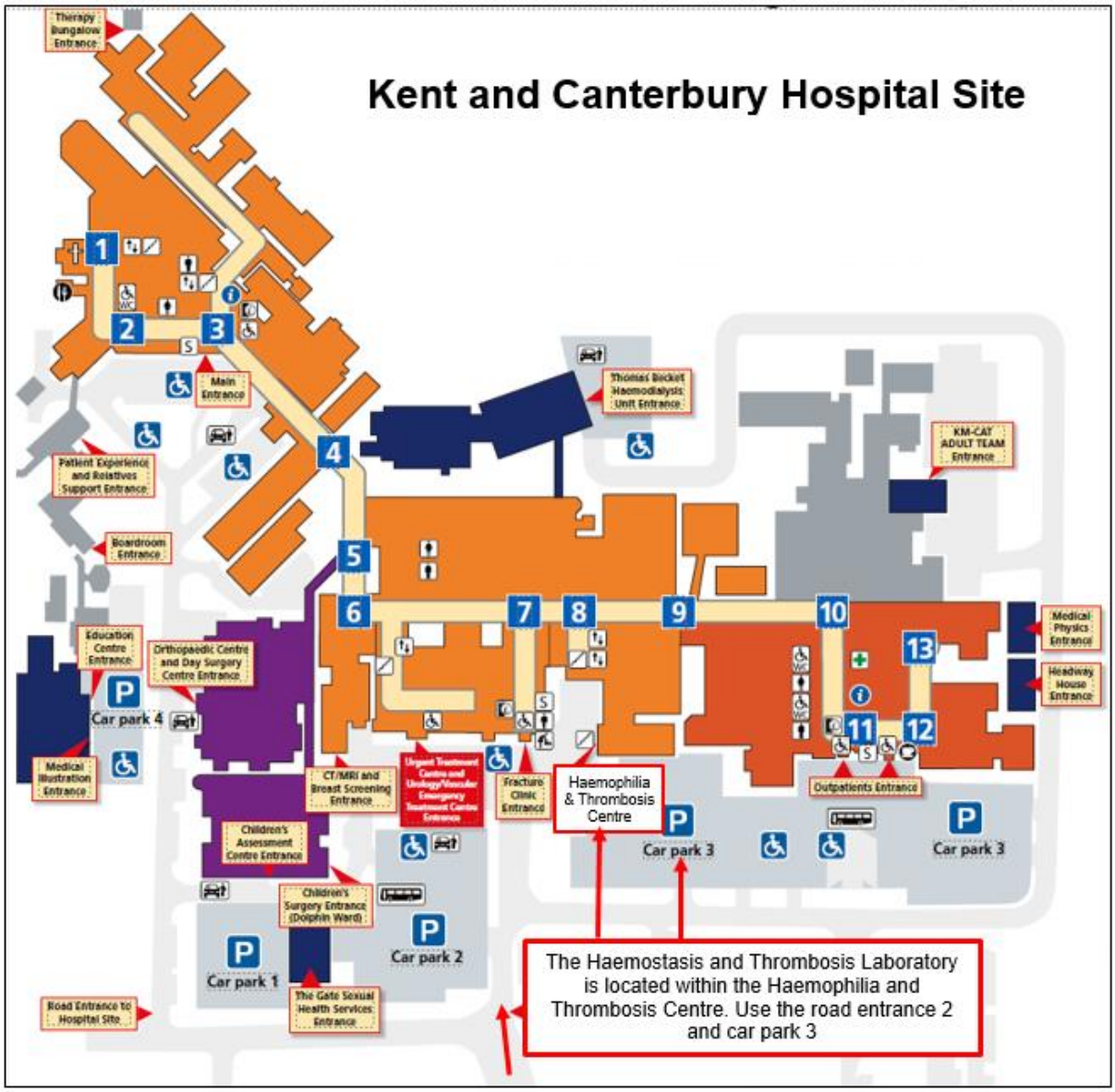
The Haemostasis and Thrombosis Laboratory is located within the Haemophilia and Thrombosis Centre based at the Kent and Canterbury Hospital site.

The Haemostasis and Thrombosis Laboratory is accessed via the Haemophilia and Thrombosis Centre situated at the rear of car park 3. If driving to the hospital use the South Canterbury Road site entrance, and follow the signs to car park 3. The car park is barrier controlled and operates a pay-on-foot system. On approaching the entry barrier, press the button to take a ticket, and pay at the station located to the left of the Outpatients building before returning to your vehicle.

If you are a blue-badge holder and park in car park 3, you will be required to pay the exit fee before leaving the hospital. However designated disabled bays for the exclusive use of blue-badge holders at no charge, can be found outside of all the main hospital entrance points. The nearest disabled parking bays to the Haemophilia and Thrombosis Centre are located outside of the Outpatients Department. Please refer to the car parking information on the EKHUFT website for full details.

On entering the Haemophilia and Thrombosis Centre, report to main reception and they will inform the Laboratory of your arrival.

Figure 1: A Map of the Kent and Canterbury Hospital Site Showing the Location of the Haemophilia and Thrombosis Centre



3 OPENING HOURS

3.1 Routine Coagulation Screening Service

A 24/7 routine coagulation screening service is provided by the Blood Science Laboratories on all acute sites across the Trust.

3.2 Specialist Coagulation Service:

The Haemostasis and Thrombosis Laboratory is open Monday to Friday between 8am and 6pm for specialist coagulation investigations.

Urgent specialist coagulation investigations can be arranged outside of routine hours by contacting the on call Haemophilia Consultant or Specialist Registrars via the hospital switchboard.

4 CONTACT NUMBERS AND KEY PERSONNEL

The main hospital switchboard number is: 01227 766877

If calling from outside the hospital, dial the main switchboard number and then once prompted add the appropriate extension number as below.

If calling from within the hospital then dial the extension number directly.

Contact	Position	Extension Number
Haemostasis and Thrombosis Laboratory (Haemophilia)		Ext. 722 5135 or 722 6329 Direct dial: 01227 866329
Dr David Gurney	Head Biomedical Scientist	Ext 722 5122
Ms Sarah Clarke	Chief Biomedical Scientist and Quality Lead	Ext. 722 3024
Dr Gillian Evans	Director of the Haemophilia and Thrombosis Centre	Ext. 722 5137
Dr Kim Elliott	Clinical Head of Service, Haemostasis and Thrombosis Laboratory	Ext. 722 8623
Dr Catherine Roughley	Consultant Haematologist	Ext. 722 8608
Dr Himali Gunasekara	Locum Consultant Haematologist	Ext. 722 3122
Haematology Specialist Registrars	On call Specialist Registrar (24/7)	01227 766877 (via the hospital switchboard).
Clinical advice: From within East Kent Hospitals:	Please use Careflow.	This is regularly monitored by the clinical team and is the quickest way to obtain urgent and routine clinical advice.
Clinical advice: From outside the Hospitals (primary care)	Please use Advice and Guidance.	This is regularly monitored by the clinical team and is the quickest way to obtain urgent and routine clinical advice.

5 CONFIDENTIALITY AND THE PROTECTION OF PERSONAL INFORMATION

Patient identifiable personal data including clinical details and past medical history is required by the Haemostasis and Thrombosis Laboratory to enable the accurate interpretation of test results and to deliver the best possible specialist coagulation services. The Laboratory follows the Trust's Data Protection, Information Governance, and Information Security Policies to ensure all identifiable personal data remains confidential, is processed fairly, lawfully and transparently, is stored securely and is only shared for lawful and appropriate purposes.

Information governance training is mandatory for all staff, to ensure they understand their individual responsibilities and obligations to respect the confidentiality of all service users, and are able to comply with the procedures in place to process identifiable personal data. This training is included in the induction procedures for all new staff and then renewed annually.

If further information is required on how the Laboratory, handles and processes confidential patient data, please contact us directly, using the contact details in Section 5 above. Further information on patient confidentiality is also available on the Freedom of Information page on the EKHUFT website.

6 COMPLAINTS AND COMPLIMENTS

6.1 Complaints

The Haemostasis and Thrombosis Laboratory is committed to offering high quality specialist coagulation services that meet and respond to the needs of all service users.

If something has gone wrong or you are not happy with any aspect of our services then please do let us know. The Laboratory follows the Trust's Complaints Management Policy, and in all cases our aim is to ensure that complaints and concerns are resolved quickly and thoroughly with appropriate investigation and resolution. The lessons learned and trends identified through complaints play a key role in improving the quality of the Laboratory service we provide.

There are a number of ways that you can make a complaint or raise a concern:

i. **Contact the Laboratory Directly**
Contact the laboratory directly either by telephone, email or in writing as below.

Telephone the Laboratory on:
Direct dial: 01227 866329 or
01227 766877, ext. 722-5135

Ask to speak to the Head or Chief Biomedical Scientist.

E-mail the Head or Chief Biomedical Scientists on:

Head Biomedical Scientist: david.gurney3@nhs.net

Chief Biomedical Scientist: Sarah.Clarke26@nhs.net

Write to the Laboratory at:
Head Biomedical Scientist
Haemostasis and Thrombosis Laboratory
Kent and Canterbury Hospital
Ethelbert Road

Canterbury
Kent,
CT1 3NG

Direct contact with the Laboratory is the best way to make a complaint as it means that we can quickly understand the problem and take immediate action to investigate and resolve the situation. If we are unable to be resolve the issue locally, then we will pass it on to the Patient Advice and Liaison Service (PALS) to further management of the complaint.

ii. Contact the Patient Advice and Liaison Service (PALS)
Complaints can also be made directly via the PALS Team on the contact details below.

Telephone: 01227 783145 or 01227 864314

E-mail: ekh-tr.PALS@nhs.net or ekhuft.complaints@nhs.net

On the Website: Complete a PALS Enquiry or Complaint Form on the EKHUFT website.

The PALS team acknowledge receipt of any complaints and pass them onto the relevant Care Group or Head of Service for investigation within 5 working days. If the concerns remain unresolved, the PALS team will pass the issue to the Trust's Complaints Team for a formal investigation

iii. Contact the Trusts Complaints Team.
Complaints can also be made directly with the Trusts Complaints Team by writing to the address below. This process is usually reserved for formal complaints which haven't been resolved locally by contact with the Head of Service or PALS.

The Complaints Team
East Kent Hospitals University NHS Foundation Trust
Trust Offices
Kent and Canterbury Hospital
Ethelbert Road
Canterbury
CT1 3NG

6.2 Compliments

We would also be delighted to hear from you if you want to tell us about something we have done well, pass on a compliment or have any suggestions on how we can improve our service. Again you can get in touch with us directly in the Laboratory by telephone, e-mail or letter using the contact details stated above.

Compliments can also be raised by completing a Compliment Form, accessed from the PALS or Patient Voice and Involvement Team pages on the EKHUFT website.

7 CLINICAL INFORMATION

It is particularly helpful to us to receive as much clinical information as possible on both electronic and hardcopy request forms as this ensures that the appropriate diagnostic tests are performed on your behalf and the correct clinical interpretation provided.

8 CLINICAL ADVICE AND INTERPRETATION

Clinical advice and interpretation is available on request from the key medical personnel listed on page 6 above. Clinical and interpretative comments are also added to the result reports if indicated

9 CONSENT FOR BLOOD TESTS

In order to take a patient's blood sample it is necessary to obtain the patient's consent. It is the responsibility of the clinician requesting the sample to ensure the patient understands the reason for making the request, the range of tests that may be involved, and the requirement to share personal data and clinical information as required for the processing and interpreting of the test results.

Patients can give consent orally or in writing, or they may imply consent by complying with the proposed examination or treatment, for example, by rolling up their sleeve to have their blood sample taken.

The Haemostasis and Thrombosis Laboratory assumes that, on receipt of a clinical sample and a completed request form, consent has been obtained by the referring clinician.

There are specific consent requirements for genetic tests. Please see Section 18.5 below for these requirements.

10 LABELLING OF REQUEST FORMS

Please help us to help you by completing request forms (electronic or conventional forms) with all the necessary information. It is essential that the patient details are clear and accurate and also that we have a clear indication of the destination for the report and the requestor.

Requests forms for haemostasis and thrombosis tests **must** include the following information:

- Full patient name, date of birth and the NHS number or hospital number
- Patient category (NHS or private patient)
- Requesting clinician
- Address/location for the report
- Relevant clinical information including anticoagulant therapy
- Date and time the sample was collected
- The test request

It is particularly important that you indicate whether the patient is on any form of anticoagulation (see section 15 below) as this will affect the interpretation of most haemostasis and thrombosis tests

11 LABELLING OF SPECIMENS

The Haemostasis and Thrombosis Laboratory adheres to East Kent Hospitals Trust Pathology Sample and Request Form Acceptance Policy.

Samples **MUST** never be pre labelled and must always be labelled in the presence of the patient.

In all cases samples MUST be clearly labelled without amendments and contain three points of patient identification as below:

1. Surname or agreed coded identifier (e.g. GU patients)
2. First name or agreed coded identifier (this must be the full name – initials are not acceptable)
3. Date of birth and/or NHS number/hospital number

It is also highly desirable for the sample tube to be labelled with the:

4. Date and time of sample collection
5. The initials of the person collecting the sample (to permit full traceability of the sample collection procedure, and provide a point of contact in the event of any labelling issues or problems).

We are not able to process any samples without the required three points of patient identification (1-3 above). This measure is in place both for the safety of patients and for the protection of Laboratory staff. The only exception to this will at the discretion of the Head of Service for very precious samples that cannot be easily repeated.

We may also reject samples that do not contain the date and time of collection. This is because for optimal sample integrity, most samples for routine and specialist coagulation assays must be analysed or separated and frozen within six hours of collection.

In addition knowledge of the sample collection time is essential for correct clinical interpretation when monitoring drug levels such as low molecular weight heparin (LMWH), rivaroxaban and apixaban, and the post treatment response in patients receiving blood products.

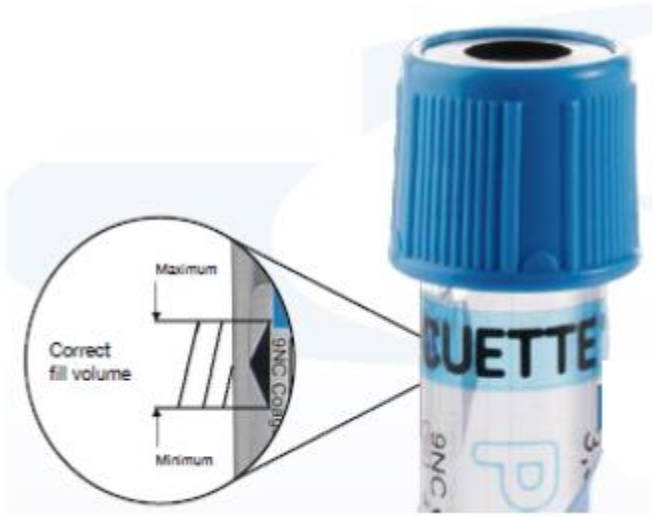
An appropriate comment will always be entered onto the final report stating why a sample has been rejected. If the requested tests are urgent, then laboratory staff will notify the requesting ward/location or clinician by telephone.

12 SAMPLE REQUIREMENTS

The majority of coagulation assays require blood to be collected into blue top, sodium citrate tubes. The blood should be collected using minimal venous stasis to ensure a good quality sample. Difficult venepuncture often results in the specimen being haemolysed, activated or clotted. Under these circumstances the results cannot be interpreted with confidence and therefore the samples will not be processed.

It is important that the specimen bottle is filled appropriately. A fill line in the form of an arrow tip (see figure 2 below) clearly indicates the minimum and maximum fill line as well as the nominal fill level. Under or over filling will produce spurious results and such samples will not be tested.

Figure 2: Coagulation Sample Showing the Maximum and Minimum Fill Lines



NB: It has been observed that when collecting coagulation tubes as the first sample using the butterfly needle collection system the tubes may not always fill to the minimum line causing them to be rejected by the Laboratory. To avoid this please select a butterfly needle with the shortest possible tubing and allow the tubing to fill with blood and displace the air before inserting and filling the coagulation tube. If you experience any problems collecting or filling coagulation tubes then please contact the Haemostasis and Thrombosis Laboratory to discuss.

Two sizes of sodium citrate coagulation tubes are available within East Kent Hospitals Trust:

- Adult tubes - requiring 3.0 ml of blood.
- Paediatric tubes - requiring 1.3 ml of blood (please note we can only perform a limited number of tests on such a small volume of blood)

For full details of the specific adult and paediatric sample requirements for our complete repertoire of coagulation assays, please see Sections 23 and 24 below.

13 TRANSPORT OF SAMPLES

- All samples transported from within the hospital should be placed at the appropriate collection point.
- Please note that the sample bags should not be used more than once.
- Arrangements should be made for any urgent samples to be transported to the Haemostasis and Thrombosis Laboratory directly.
- Samples for specialist coagulation assays can be delivered to the Pathology Laboratories at any of the hospital sites across the Trust for onward transport to the Haemostasis and Thrombosis Laboratory. Specific Versapak insulated transport boxes are provided to transport Haemostasis and Thrombosis samples between sites.
- All samples for coagulation assays must reach one of the Laboratories across the Trust within six hours of sample collection, or be centrifuged, separated and frozen for transport at a later date.
- Frozen samples must be transported in an appropriate container and outer transport box which ensures that they remain completely frozen throughout the whole journey.
- If required, the Laboratory can provide advice on suitable containers, labels and boxes for the transport of samples.

14 URGENT REQUESTS

- Please request tests to be performed urgently only when it is clinically essential.
- All of our work is processed rapidly and the results are available in a timely manner. The agreed turnaround times for each test are published at the end of this user guide.
- If you wish for a sample to be analysed urgently, please make sure that the request clearly states this and always contact the laboratory in advance to discuss your requirements.
- If the phlebotomist bleeds the patient, please ensure that the phlebotomist understands that the sample is urgent and needs to be transported immediately to the Haemostasis and Thrombosis Laboratory.
- These samples will be handled separately and the results telephoned to the requesting doctor as soon as possible.
- Critical routine coagulation results produced in the Blood Sciences Laboratories at the Queen Elizabeth the Queen Mother (QEQM) and William Harvey Hospital (WHH) sites will be communicated to the respective Accident and Emergency Departments using the PTL system.

15 TELEPHONED RESULTS

- Please avoid asking us to telephone results if possible as this interferes with the work of the laboratory
- The Haemostasis and Thrombosis Laboratory and the routine coagulation screening services based on each acute site have an agreed list of critical/alert results that will always be telephoned to the ward and/or requesting clinician as shown in the table below.

Haemostasis and Thrombosis Laboratory Telephone Alert Ranges

Telephone the Requestor Urgently	Inform the Haemophilia Centre Consultant / Registrar
Any INR **≥ 8.0**	
Any patient receiving unfractionated heparin and APTT ratio is **<1.5 or >2.5**	
Any first time unexpected, grossly prolonged PT or APTT result and the patient is **not** on anticoagulants. **PT >25 seconds or APTT >50 seconds.**	
Any anti-Xa level **>1.0 U/mL or <0.1 U/mL**	
Any fibrinogen **<1.0 g/L**	
Any request form stating DIC or ?DIC on the clinical details if the fibrinogen is **<1.5 g/L**, or the **PT or APTT are >5 seconds** above the normal range, or if the patient is bleeding.	
Any patient on direct thrombin inhibitors (such as Dabigatran, Bivalirudin and Argatroban) or direct factor Xa inhibitors (such as Rivaroxaban, Apixaban and Edoxaban) if the request form states “bleeding”.	

Telephone the Requestor Urgently	Inform the Haemophilia Centre Consultant / Registrar
Any patient with abnormal coagulation results and the request form states “bleeding”.	
Any patient on thrombolytic therapy and the request form states “bleeding”.	Any patient on thrombolytic therapy and the request form states “bleeding”.
	Any newly diagnosed coagulopathies: • Discuss with Haemophilia consultant. • Refer to Haemophilia Consultant Queue if non urgent.
	Any newly diagnosed inhibitors: Inform Haemophilia consultant urgently.

- We will always ask you to confirm any results that we do give you by telephone by reading both the test name and the results back to us.
- We will always ask for the full name of the person taking the results for audit purposes.
- The above protocol will also be applied if you telephone the laboratory for results.

16 HIGH RISK SAMPLES

The Laboratory operates a policy of universal safety precautions for all samples and we recommend that you regard all blood as being potentially infectious. High risk labelling of samples is not required.

17 UKAS ISO15189 ACCREDITATION:

The Haemostasis and Thrombosis Laboratory service is accredited by UKAS in conformance with the ISO 15189 Standards. Our UKAS Medical Laboratory Reference Number is 9397.

Our aim is to have all our tests accredited to the ISO 15189 Standards, but there are a small number of esoteric tests that are not UKAS accredited, and a small number of newly introduced tests, and tests that have been implemented on upgraded analysers that are currently undergoing an extension to scope application to be accredited. All none accredited tests or those pending an extension to scope are clearly indicated by a comment on the final report.

For a full list of our accredited tests, please click on the link below to view our Schedule of Accreditation on the United Kingdom Accreditation Service (UKAS) website.

9397 Medical Single 003 HL (ukas.com)

18 ROUTINE TESTS

18.1 Routine Coagulation Screen

The Laboratory recommends the following as a routine coagulation screen:

- Prothrombin Time (PT)
- Activated Partial Thromboplastin Time (APTT)

Additional screening tests including a fibrinogen assay, thrombin time and Reptilase time will be performed by the laboratory as indicated by the clinical situation and the results of the PT and APTT.

Please notify the laboratory on the request (electronic or hardcopy) if a patient is known to be taking any type of anticoagulant drug (e.g. warfarin, heparin, LMWH, rivaroxaban, apixaban, edoxaban and dabigatran etc.) which may affect coagulation testing.

18.2 D-Dimer

The D-Dimer should only be used in clearly defined circumstances. These are:

- Diagnosis of venous thrombosis [deep vein thrombosis (DVT) and pulmonary embolism (PE)] **only** when performed alongside a clinical probability score. It is important to note that a positive D-Dimer does not confirm the diagnosis. Nor does a negative D-Dimer in isolation exclude a DVT or PE but should be taken into consideration with other clinical features. It is also important to note that the D-Dimer may be non-specifically raised in other conditions such as cancer and infection.
- Where there is clinical suspicion of disseminated intravascular coagulation (DIC)

In order to support the consistent application of these criteria by laboratory and clinical staff across the Trust there is a Trust policy for the use of the D-Dimer assay. This is available in the Policy Centre on the Trust Intranet and outlines the clinical situations when it is appropriate and inappropriate to perform a D-Dimer assay. These are also listed below.

- D-dimers **will be** available for the investigation of patients presenting with possible venous thrombosis, as long as the request for the test is accompanied by the **clinical probability (Wells) score** and an **applicable diagnosis**.

- D-dimers **may** be requested for the investigation of DIC but will be performed at the discretion of the laboratory, depending upon the initial coagulation screen results.

- D-dimers will **not be** available for established in-patients as a significant majority will already have raised D-dimer levels due to concurrent illness.

- D-dimers **will not** be available for the investigation of arterial thrombosis (i.e. for the investigation of a heart attack or stroke).

- D-dimers **are not** indicated in patients presenting with symptoms such as collapse, chest pain, dyspnoea and headache, unless a pulmonary embolus is suspected.

- D-dimers **should not** be performed on patients already receiving anticoagulants.

18.3 Anticoagulant Therapy

18.3.1 Anticoagulant Therapy with Oral Vitamin K Antagonists

- The doctors on the wards at the three acute hospital sites across the Trust are responsible for monitoring and dosing all inpatients on oral anticoagulant therapy with vitamin K antagonists (Warfarin and Sinthrome).
- The Haemostasis and Thrombosis Laboratory provides an inpatient anticoagulant monitoring and dosing service for the three local cottage hospitals; Faversham Cottage Hospital, Queen Victoria Memorial Hospital and Whitstable and Tankerton Hospital. The Laboratory will only accept patients for dosing if an appropriately completed anticoagulant referral form (electronic or paper) is received.
- The International Normalised Ratio (INR) is used to monitor patients on oral anticoagulant therapy with vitamin K antagonists.

- The INR is meaningless in patients who are not receiving oral anticoagulants and will not be reported as part of a routine coagulation screen assessment. The only other time that the laboratory will issue an INR result is for patients who have taken a paracetamol overdose or have liver disease and may need to be referred to the Liver Unit at King's College Hospital.

18.3.2 Unfractionated Heparin Therapy

- Therapeutic treatment with unfractionated heparin is monitored using the activated partial thromboplastin time ratio (APTTr).
- The sample **must** be received in the laboratory and analysed within two hours of sample collection. This is because the release of platelet factor 4 from platelets in vitro progressively neutralises any heparin in the sample resulting in an underestimation of the drug level.
- Please contact the Haemophilia Centre Medical staff if you require advice regarding the control and monitoring of unfractionated heparin therapy.

18.3.3 Low Molecular Weight Heparin Therapy (LMWH)

- Patients on LMWH do not generally require laboratory monitoring except under certain clinical circumstances (for example in children, during pregnancy and in patients who are obese, have renal disease or are bleeding).
- The anti-Xa assay is used to monitor LMWH therapy. The sampling time is very important and for peak concentrations samples **must** be collected 4-6 hours after the last LMWH injection.
- Samples for monitoring LMWH by the anti-Xa assay **must** be received in the laboratory and analysed, or separated and frozen within two hours of collection. This is because the release of platelet factor 4 from platelets in vitro progressively neutralises any heparin in the sample leading to spuriously low levels.

18.3.4 Direct Oral Anticoagulants (DOACs)

- Particular attention should be given to patients on the direct acting oral anticoagulants such as the direct thrombin inhibitors (e.g. Dabigatran) and the direct factor Xa inhibitors (e.g. Rivaroxaban, Apixaban and Edoxaban).
- It is essential that clinical information is provided identifying patients on these drugs to prevent unnecessary investigations and the incorrect interpretation of results.
- Anti-Xa assays for measuring the plasma concentration of both rivaroxaban and apixaban are available in the Laboratory. Routine monitoring of these drugs is not indicated. However for patients in whom surgery is planned or who are bleeding, please contact the Haemostasis and Thrombosis Laboratory to discuss appropriate testing.

18.4 Specialist Coagulation Investigations

18.4.1 Investigation of Bleeding Disorders

- A range of specialised coagulation investigations are available for patients with suspected bleeding disorders such as haemophilia or von Willebrand Disease.
- These patients should be referred to the Haemophilia Centre where the appropriate samples can be taken.
- Platelet function testing is only available after discussion with the Haemophilia Centre Medical staff and needs to be prearranged and booked with the Haemostasis and Thrombosis Laboratory.

18.4.2 Thrombophilia and Antiphospholipid Investigations

- National Guidelines exist on the indications for thrombophilia screening and these are followed by the Haemostasis and Thrombosis Laboratory and are incorporated in to the Trust Guidelines for Thrombophilia Testing which are available in the Policy Centre on the Trust Intranet.

- Results of thrombophilia and antiphospholipid antibody investigations may be affected by patient variables such as acute thrombosis, pregnancy, and anticoagulation therapy and therefore should not be routinely tested for in these situations.
- Testing is also generally not advised in children under the age of sixteen years

18.5 Molecular Genetics Investigations

18.5.1 In-House Molecular Genetics Service

The Haemostasis and Thrombosis Laboratory runs a small in-house molecular genetics service for the detection of the Factor V Leiden G1691A and the Prothrombin Gene G20210A mutations. Both tests are performed on the GeneXpert analyser which integrates sample purification, nucleic acid amplification, and detection of the target sequence in whole blood using real-time polymerase chain reaction (real-time PCR) assays.

A minimum of 1 ml of EDTA whole blood is required for Factor V Leiden G1691A and Prothrombin Gene G20210A mutation analysis. A single sample is sufficient for both tests but to avoid the possibility of DNA contamination a separate, virgin sample, specific for genetics analysis **must** always be collected.

As with other causes of inherited thrombophilia, testing for the Factor V Leiden G1691A and Prothrombin Gene G20210A mutations is not generally advised in children under the age of sixteen years. Any possible requirement to test a child must always be discussed with one of the haemophilia consultants before proceeding.

18.5.2 Referred Molecular Genetic Tests

Genomics tests for rare and inherited diseases are now commissioned and funded through NHS England and Improvement, and provided through a national testing network consolidating and enhancing the existing laboratory provision. The South East Genomics Laboratory Hub, a network of leading foundation trusts and pathology providers led by Guys and St Thomas' NHS Foundation Trust, is our local Genomics Laboratory Hub and has been commissioned to deliver all genomic testing services across South London, Kent, Surrey and Sussex.

The National Genomic Test Directory specifies which genomic tests are commissioned by the NHS in England, the technology by which they are available, and the patients who will be eligible to access to a test, and can be accessed on the South East Genomics website (https://southeastgenomics.nhs.uk/).

We send all samples for genetic testing for inherited bleeding and thrombotic disorders to the Molecular Haemostasis and Thrombosis Laboratory, Synnovis, St Thomas' Hospital, which is part of the South East Genomics Laboratory Hub.

18.5.3 Consent for Genetic Testing

All genetic testing requires informed consent, including requests for Factor V Leiden G1691A and Prothrombin Gene G20210A mutation analysis received from primary care, and requested as part of a thrombophilia screen. It is the responsibility of the clinician requesting genetic analysis to ensure the patient understands the reason for making the request, the potential implications of the results, and the requirement to share personal data and clinical information as required for the processing and interpreting of the test results.

The laboratory assumes that, on receipt of a clinical sample and a completed request form, consent has been obtained and recorded by the referring clinician.

Requests for genetic testing, other than for Factor V Leiden G1691A and Prothrombin Gene G20210A mutation assays, will only be accepted following discussion and agreement with one of the Haemophilia consultants, and in most cases this requires referral of the patient to

the Haemophilia and Thrombosis Centre for review. If a patient attends the Haemophilia and Thrombosis Centre for agreed genetic testing, then the Haemophilia Clinical Team will provide the required information to the patient and obtain informed consent for testing.

For further information on the molecular genetic tests available or to discuss your specific requirements, please contact the Haemostasis and Thrombosis Laboratory on:

Tel: 01227 766877, Ext. 722-5135, or 01227 866329

For clinical genetics advice, please contact the Clinical Team as below:

- **From within East Kent Hospitals:** Please use Careflow. This is regularly monitored by the clinical team and is the quickest way to obtain urgent and routine clinical advice.

- **From outside the Hospitals (primary care):** Please use Advice and Guidance. This is regularly monitored by the clinical team and is the quickest way to obtain urgent and routine clinical advice.

19 UNCERTAINTY AND FACTORS AFFECTING COAGULATION RESULTS

Measurement uncertainty provides a way of assessing the variability in results that the laboratory would normally expect if an assay were to be repeated another time. Many factors can affect the results of coagulation tests and their clinical interpretation and these are usually grouped according to where they occur in the pre examination (request), examination (test) and post examination (report) cycle. These are summarised in figure 3 and discussed in more detail below.

If you would like further information or require an uncertainty of measurement (UOM) value for each test, please contact the Laboratory directly using the contact details in Section 4 above and we would be very happy to provide this.

Figure 3: Sources of Uncertainty in the Request-Test-Report Cycle.

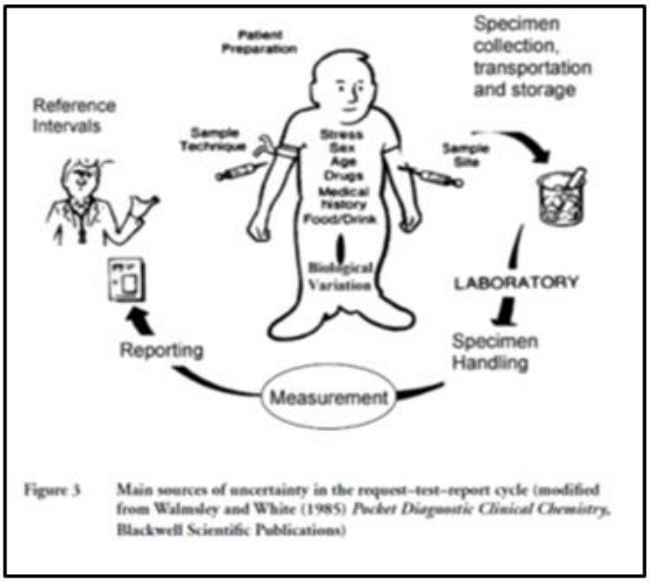


Figure 3 Main sources of uncertainty in the request–test–report cycle (modified from Walmsley and White (1985) *Pocket Diagnostic Clinical Chemistry*, Blackwell Scientific Publications)

19.1 Pre Examination Factors Affecting Coagulation Results

All coagulation results will be subject to variability arising from how the sample is collected and stored. Differences in patient preparation, specimen collection technique, time of sampling, transportation, storage time and preparation of the primary sample may all alter the results and the measurable amount of an analyte in a sample. Other factors that may influence coagulation results are generally patient specific and include stress, jaundice, underlying clinical conditions and certain drug therapies.

As users of the Haemostasis and Thrombosis Laboratory Service you play a key role in reducing the effects of pre analytical variables on coagulation results by following the information and advice provided in this Users Guide to ensure that you collect a good quality sample at the appropriate time and for the appropriate tests. There are a number of steps that you can take to ensure the quality of the sample that you send to us:

- Always check the sample requirements, particularly for special coagulation investigations.
- Always check the timing requirements for the sample particularly if monitoring drug therapy such as LMWH, rivaroxaban, apixaban and edoxaban.
- Ensure the samples are taken in the correct order of draw – **1.** Blood culture or no additive tubes, **2. Coagulation tubes**, **3.** Serum tubes with/without gel, **4.** Heparin tubes with/without gel, **5.** EDTA tubes, **6.** Glucose tubes and **7.** Other tubes
- Do not take the sample from an arm with a drip.
- Do not tip blood from one bottle to another, as this will result in an incorrect blood to anticoagulant ratio or may contaminate the sample with an inappropriate anticoagulant
- Samples **must** be filled exactly to the level indicated on the bottle. Overfilled and under filled samples are unsuitable for analysis.
- As soon as the sample is in the bottle, mix thoroughly by gentle inversion. Do not shake.
- Ensure the samples are delivered promptly to the laboratory.
- Samples > 2 hours old when they arrive in the laboratory are unsuitable for unfractionated heparin or LMWH monitoring and will be rejected.
- Samples >6 hours old when they arrive in the laboratory are unsuitable for all testing and will be rejected.
- If the patient is on any type of anticoagulant, please state this clearly on the request form.

19.2 Examination Factors Affecting Coagulation Results

As with all examination procedures there are numerous analytical factors that may introduce variability into the results of our coagulation assays. These include uncertainty of the calibrator value and dispensed volumes, reagent and calibrator batch variations, equipment maintenance and age, different operators, and environmental fluctuations. There may also be substances present in the sample that interfere with the test procedure such as certain drugs or bilirubin. The laboratory pays careful attention to these factors and takes a range of steps to minimise their effects on results including:

- Where available all tests are referenced to and calibrated against a traceable reference material.
- Following national guidelines and protocols.
- Annual commercial service and calibration of all laboratory pipettes and the laboratory balance and regular ongoing in-house calibration checks.
- A comprehensive internal and external quality control programme with careful monitoring of the accuracy, precision and bias of all assays.
- Strict adherence to standard operating procedures and manufacturer's maintenance schedules.
- Regular competency assessment of all staff.
- Assessing the limitations, interfering substances and cross reactions affecting all assays.

- Calculation of an uncertainty of measurement (UOM) value for each quantitative test based on the bias and imprecision (randomness) of the assay system.

19.3 Post Examination Factors Affecting Coagulation Results

A number of factors can affect the interpretation of routine and special coagulation assays. Some assays produce raw numerical data that is then manipulated to produce a final result, and it is possible for calculations to introduce errors (e.g. rounding up numbers) and lead to variability of results. Disease and physiological factors such as biological variation, stress and pregnancy can all bring uncertainty to the interpretation of results. If the result is distinct from the clinical decision value then these factors are generally of little or no importance but as results approach clinical decision values they may significantly affect interpretation. The table below summarises the important factors that can affect the results and interpretation of routine and special coagulation assays, and which should be considered when reviewing patient results. To help our users with this we add clinical and interpretative comments to our reports if indicated.

Test	Indication	Variables Affecting the Results	Comments
Routine Coagulation Screen (PT & APTT)	Initial evaluation of haemostasis	Anticoagulant therapy (e.g. heparin, LMWH, warfarin, direct thrombin and Xa inhibitors), and underlying clinical conditions such as Lupus anticoagulant	• It is essential that appropriate clinical details are provided to prevent unnecessary further investigations and incorrect interpretation of results.
D-Dimer	Investigation into possible venous thrombosis or DIC	Increasing age, pregnancy and underlying clinical conditions	• Is non-specifically raised in inflammatory conditions, infections and malignancy
Anti-Xa Assay LMWH	Monitoring low molecular weight heparin (LMWH) therapy	Timing of sample	• For peak levels, the sample must be taken 4-6 hours post last injection of LMWH. • Sample must be analysed or separated and frozen within two hours of collection.
Anti-Xa Assay Rivaroxaban Anti-Xa Assay Apixaban	Measurement of Rivaroxaban levels Measurement of Apixaban levels	Timing of sample	• For peak plasma levels, the sample must be taken at 2-3 hours post ingestion of rivaroxaban or apixaban. • The date and time of rivaroxaban or apixaban tablet ingestion must be stated on the request form
Lupus Anticoagulant Testing	Investigation of possible antiphospholipid syndrome	An acute event such as thrombosis or stroke, anticoagulant therapy (e.g. heparin, LMWH, warfarin, direct thrombin and factor Xa inhibitors), and pregnancy.	• False positive results may be obtained in patients on anticoagulant therapy. • Unreliable results may be obtained during an acute event such as a thrombosis or stroke and during pregnancy. • Do not test during pregnancy.

Test	Indication	Variables Affecting the Results	Comments
			• Defer testing until 12 weeks post an acute event. • Defer testing if possible until patient is off anticoagulants.
Thrombophilia Testing	See BSH guidelines	Acute thrombosis, pregnancy, oral contraceptives and anticoagulant therapy.	• Do not test during pregnancy or in acute thrombosis. • Defer testing if possible until patient is off anticoagulants.
Factor VIII and von Willebrand Factor Assays	Testing for haemophilia A or von Willebrand disease	Pregnancy, oral contraceptives, hypothyroidism, and acute phase response (stress and inflammation)	• Interpret tests with caution in these scenarios.
Platelet function testing	Investigate possible bleeding tendency	Anti-platelet drugs including non-steroidal anti-inflammatory drugs (NSAID)	• Interpret tests with caution in patients on anti-platelet therapy or defer testing until therapy stopped. • Ensure fully completed pre-venepuncture questionnaire is provided.
Protein C	Investigation of possible Protein C deficiency	Pregnancy, post-partum, combined oral contraceptives (COC), age and anticoagulant therapy	• Can be falsely normal in pregnancy, post-partum and in patients on COC. • Levels may not reach normal range until late teens. • Defer testing until anticoagulant therapy stopped
Protein S	Investigation of possible Protein S deficiency	Pregnancy, COC therapy, and anticoagulant therapy	• Can be falsely low in pregnancy and in patients on COC • Defer testing until anticoagulant therapy stopped.

20 REPORTS

As soon as they have been authorised, results will be available to view electronically on Sunrise for internal users within EKHUFT and on DART OCM for external users in the community. Hardcopy reports will continue to be issued to external users who do not have access to DART OCM, but the Laboratory is moving away from issuing hardcopy reports and the ultimate aim is paperless reporting.

Reference ranges are periodically re-evaluated and can be found on the paper and electronic report alongside each result. If a reference range has been recently altered a comment will be placed below the test for a period of six months to indicate this.

21 SAMPLES REFERRED TO OTHER TRUSTS FOR ANALYSIS:

There are a small number of low volume esoteric tests that it is not cost effective to perform in the Haemostasis and Thrombosis Laboratory and these are referred to specialist laboratories outside of the East Kent Hospitals Trust. The Haemostasis and Thrombosis Laboratory ensures that where possible each referral laboratory has full UKAS Accreditation and participates in a recognised external quality assessment scheme for each referred test and this status is checked regularly.

Samples will be sent off to the referral laboratory using appropriate postal or courier methods and the Haemostasis and Thrombosis Laboratory will manage the dispatch and return of results process. A procedure for monitoring the turnaround times of these samples is in place. Where possible reports will be sent out using similar mechanisms used for internal processing; however, the reports will always contain the name and appropriate reference ranges of the processing laboratory.

The table below lists the referral laboratories that we currently use and the assays performed (for referred genetic assays, please see section 16.5.2 above). Please contact the Haemostasis and Thrombosis Laboratory to discuss sample requirements, completion of the appropriate request forms, testing procedures and turnaround times if you require any of these assays.

Referral Laboratory	Hospital	UKAS Number	Test	Accredited Test
Coagulation Laboratory	Royal Hallamshire Hospital, Sheffield	8508	Chromogenic factor IX assay	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Genetic analysis for bleeding and thrombotic disorders.	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Porcine factor VIII inhibitor assay	Not Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Anti-Xa Edoxaban	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Anti-Xa Fondaparinux	Not accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Taipan Clotting Time (TSVT)	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Flow cytometry for platelet glycoproteins	Not accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Alpha-2-antiplasmin activity	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	von Willebrand factor (VWF) multimers	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Emicizumab insensitive Bethesda factor VIII assay.	Not Accredited

Referral Laboratory	Hospital	UKAS Number	Test	Accredited Test
Synnovis Analytics LLP	St Thomas' Hospital	8595	vWF: FVIII binding assay	Not Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Emicizumab levels	Not Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Warfarin plasma concentration, PIVKA-II and vitamin K1	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	High Molecular Weight Kininogen	Accredited
Synnovis Analytics LLP	St Thomas' Hospital	8595	Prekallikrein	Accredited
Haematology Laboratory	Tunbridge Wells Hospital	8342	Derived Fibrinogen	Not Accredited
HSL Analytics LLP	Haemophilia Laboratory, Royal Free Hospital	9345	Platelet Nucleotide assay	Accredited
HSL Analytics LLP	Haemostasis Laboratory, the Halo Building	10204	ADAMTS-13 Activity	Accredited
HSL Analytics LLP	Haemostasis Laboratory, the Halo Building	10204	IgG anti platelet factor 4 antibodies (VITT)	Accredited

Please contact us directly for the specific turnaround times for each referred test. These are determined by the referral laboratory performing the assay, with a small amount of additional time to allow the Haemostasis and Thrombosis Laboratory to enter and report the results.

22 TIME LIMITS FOR REQUESTING ADDITIONAL EXAMINATIONS

Due to the deterioration of liable clotting factors, there is a time limit on requesting additional examinations. Six hours after the original sample was taken, we will be unable to add additional examinations to the sample as the integrity of the sample may have become compromised.

23 TEST REPERTOIRE AND ADULT SAMPLE REQUIREMENTS

The Haemostasis and Thrombosis Laboratory offers an extensive test repertoire as listed in the table below but if you cannot see the test that you require then please always contact us to discuss and we will do our best to help.

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
Routine Coagulation Tests • **Coagulation screen**: Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) • **INR** for monitoring oral anticoagulant therapy • **APTT ratio** (APTTr) for monitoring heparin therapy **NB: Fibrinogen, Reptilase and Thrombin Time (TT)** tests will automatically be added by the laboratory if indicated by the clinical details and the results of the routine coagulation screen. **No additional samples are required for these extra tests.**	1 x 3.0ml citrate tube (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send samples within the designated timescales, they should be centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples for the routine tests must either arrive in the laboratory or be separated and frozen within 6 hours of collection. • Samples for heparin monitoring must either arrive in the laboratory or be separated and frozen within 2 hours of collection.
D-Dimer:	1 x 3.0ml citrate tube (blue cap)  **NB: A coagulation screen is always performed with every D-Dimer request. One sample is sufficient for both tests.**	As for the routine coagulation tests above	• Samples must either arrive in the laboratory or be separated and frozen within 6 hours of collection. • The request must be accompanied by a clinical probability (Wells) score and an applicable diagnosis when the D-Dimer is being used to exclude venous thrombosis. See the Trust Policy on Use of the D-Dimer on the Policy Centre.

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
LMWH Anti-Xa Assay for monitoring Low Molecular Weight Heparin	1 x 3.0ml citrate tube (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within two hours of collection, they should be double centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must be collected 4-6 hours after the last LMWH injection for peak levels. • The date and time of the last LMWH injection must be stated on the request from • Samples must arrive in the laboratory or be separated and frozen within 2 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent
Rivaroxaban Anti-Xa Assay for monitoring Rivaroxaban **Apixaban Anti-Xa Assay** for monitoring Apixaban	1 x 3.0ml citrate tube (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within six hours of collection, they should be double centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• For peak plasma levels, the sample must be taken at 2-3 hours post ingestion of rivaroxaban or apixaban. • The date and time of rivaroxaban or apixaban tablet ingestion must be stated on the request from • Haemolysed plasma is not suitable for analysis and should not be sent • Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection.

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
Thromboelastography (TEG 5000)	2 x 3.0ml citrate tube (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • Samples must be tested within 2 hours of collection. If not collected in the Haemophilia Centre the samples must be delivered to the laboratory by hand immediately after collection	• **Performed only by agreement** with one of the Haemophilia consultants and must be pre-booked with the Haemostasis and Thrombosis Laboratory. • Samples must be drawn with a 21 G butterfly needle and the first bottle or first 5 ml must be discarded. The second bottle collected is used for analysis. Always write the order of draw on the collection tubes.
Factor Assays: • One stage factor assays (factors II, V, VII, VIII, IX, X, XI, and XII) • Refacto Factor VIII Assay • Factor IX Assay for Extended Half-life Products. • Factor XIII Assay	2 x 3.0ml citrate tubes (blue cap) for any combination of factor assays 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within 6 hours of collection, they should be centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
Chromogenic Factor VIII Assay	2 x 3.0ml citrate tubes (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send samples within 6 hours of collection, they should be centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Factor VIII and IX Inhibitor Assays	2 x 3.0ml citrate tubes (blue cap). This will also include the associated factor assay. 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send samples within 6 hours of collection, they should be centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
Investigations for von Willebrand Disease • Factor VIII assay • von Willebrand Factor Antigen (vWF:Ag) • Ristocetin Cofactor Activity • vWF:CBA (collagen binding activity) if required.	3 x 3.0ml citrate tubes (blue cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within 6 hours of collection, they should be centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. • Separated plasma should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Platelet Function Analysis (PFA)	By special arrangement only. Please contact the Laboratory to discuss sample requirements.	• After collection samples should be kept at room temperature. Do **not** refrigerate. • Samples are stable for four hours after collection if stored undisturbed at room temperature.	• **Performed only by agreement** with one of the Haemophilia consultants and must be pre-booked with the Haemostasis and Thrombosis Laboratory. • Samples must be received in the Laboratory and tested within 4 hours of collection. • The samples **must not** be centrifuged.
Platelet Aggregation	By special arrangement only. Please contact the Laboratory to discuss sample requirements	• After collection samples should be kept at room temperature. Do **not** refrigerate. • Samples must be tested within two hours of collection.	• **Performed only by agreement** with one of the Haemophilia consultants and must be pre-booked with the Haemostasis and Thrombosis

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
		If not collected in the Haemophilia Centre the samples must be delivered to the laboratory by hand immediately after collection.	Laboratory. • Samples must be received in the Laboratory and tested within 2 hours of collection.
Lupus Anticoagulant / Anti-phospholipid Screen (including anticardiolipin antibodies)	2 x 3.0ml citrate tubes (blue cap)  + 1 plain serum sample (red cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within six hours of collection the citrate samples (blue cap) should be double centrifuged at 2000g for 10 minutes and the plasma separated & stored at -20°C in the freezer. The serum sample (red cap) should be centrifuged once at 2000g for 10 minutes and the serum separated & stored at -20°C in the freezer. • The separated plasma/serum should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
Anticardiolipin Antibodies (Included in thrombophilia screen and Lupus Anticoagulant screen but may also be requested on its own).	1 plain serum tube (red cap) 	• After collection samples should be kept at room temperature. Do **not** refrigerate. • If it is not possible to send the samples within 6 hours of collection, they should be centrifuged at 2000g for 10 minutes and the serum separated stored at -20°C in the freezer. • Separated serum should be sent to the Laboratory at the earliest opportunity and within two weeks of being frozen.	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection.
Anti-Beta2-Glycoprotein 1 Antibodies	1 plain serum tube (red cap) 	As for anticardiolipin antibodies above.	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection.
Thrombophilia Screen: Including • Protein C • Protein S • Antithrombin • Lupus Anticoagulant • Anticardiolipin antibodies • Factor V Leiden G1691A mutation • Prothrombin Gene G20210A mutation	3 x 3.0 ml citrate tubes (blue cap)  + 1 plain serum tube (red cap)  + 1x EDTA tube for genetic analysis for the Factor V Leiden and Prothrombin mutations. 	If it is not possible to send the samples within six hours of collection then: • Double centrifuge the three citrate samples at 2000g for 10 minutes, separate the plasma and store at -20°C in the freezer. • Centrifuge the serum sample once at 2000g for 10 minutes, separate the serum and store at -20°C in the freezer. • EDTA sample. Do **not** centrifuge. Store in the fridge	• The Laboratory has specific indications for thrombophilia testing based on the BSH Guidelines. These are available in Policy Centre on the Trust Internet and if they are not met testing will not be performed. However, the samples will be saved and the requestor given the opportunity to clarify the indication for testing. • To avoid DNA contamination, the EDTA sample must

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
		at 2-8ºC and send as whole blood.	be a virgin sample that hasn't been used for any other test. • Ideally samples should arrive in the Laboratory within 2 weeks of collection.
Factor V Leiden G1691A Mutation (also included in thrombophilia screen)	1x EDTA tube (purple cap)  NB: To avoid DNA contamination, this must be a virgin sample that hasn't been used for any other test.	• Do not centrifuge. Samples can be stored at 2°- 8°C in the fridge for up to 2 weeks before analysis. After 2 weeks the sample quality begins to deteriorate.	• Ideally the samples should arrive in the Laboratory within 2 weeks of collection.
Prothrombin Gene G20210A Mutation (also included in thrombophilia screen)	1x EDTA tube (purple cap)  NB: To avoid DNA contamination, this must be a virgin sample that hasn't been used for any other test	• The primary samples can be stored at 2°- 8°C in the fridge for up to 2 weeks before analysis. After 2 weeks the sample quality begins to deteriorate.	• Ideally the samples should arrive in the Laboratory within 2 weeks of collection.
Heparin Induced Thrombocytopenia Assay (HIT)	1 plain serum tube (red cap) 	• Samples that cannot be tested immediately should be centrifuged at 2000g for 10 minutes and the serum removed • The separated serum can be kept at 4°C in the fridge for 48 hours before analysis. To store the sample for longer periods or if sending from another site, the	• Performed only by agreement with one of the Haemophilia Medical staff. • In all cases please contact the Haemostasis and Thrombosis Laboratory to discuss before sending the sample • For same day analysis, samples must reach the Laboratory before midday.

Test	Sample Requirements	Post Collection Sample Storage Requirements	Special Instructions
		serum should be frozen at -20°C in the freezer. • The separated serum should be sent to the Laboratory as soon as possible after collection	• A HIT clinical score (4T score) is required for full interpretation of the results. This must be written on the accompanying request form, or in the clinical details for electronic requests.

24 PAEDIATRIC SAMPLE REQUIREMENTS

NB: The post collection sample storage requirements for each test are exactly the same as for adult samples. Please refer to the table above for this information.

Test	Sample Requirements	Special Instructions
Routine Coagulation Screen: Including • Prothrombin Time (PT) • Activated Partial Thromboplastin Time (APTT) **NB: Fibrinogen, Reptilase and Thrombin Time (TT)** tests will automatically be added by the laboratory if indicated by the clinical situation and the results of the routine coagulation screen, and if sufficient plasma	1 x 1.3ml paediatric citrate bottle (blue screw cap) 	• Samples must either arrive in the laboratory or be separated and frozen within 6 hours of collection. • Samples for heparin monitoring must either arrive in the laboratory or be separated and frozen within 2 hours of collection.
D-Dimer **NB: A coagulation screen is always performed with every D-Dimer request. One sample is sufficient for both tests.**	1 x 1.3ml paediatric citrate bottle (blue screw cap) 	• Samples must either arrive in the laboratory or be separated and frozen within 6 hours of collection
LMWH Anti-Xa Assay for monitoring Low Molecular Weight Heparin	1 x 1.3ml paediatric citrate bottle (blue screw cap) 	• Samples must be collected 4-6 hours after the last heparin injection for a peak level. • Samples must arrive in the laboratory or be separated and frozen within 2 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent

Test	Sample Requirements	Special Instructions
Thromboelastography (TEG 5000)	1 x 1.3ml paediatric citrate bottle (blue screw cap) 	• **Performed <u>only</u> by agreement** with one of the Haemophilia consultants and must be pre-booked with the Haemostasis and Thrombosis Laboratory. • Samples must be drawn with a 21 G butterfly needle and the first bottle or first 5 ml must be discarded. The second bottle collected is then used for analysis. Please write the order of draw on the collection tubes. • Samples must be tested within 2 hours of collection. If not collected in the Haemophilia Centre the samples must be delivered to the laboratory by hand immediately after collection.
Factor Assays: • One stage factor assays (factors II, V, VII, VIII, IX, X, XI, and XII) • Refacto Factor VIII Assay • Factor IX Assay for Extended Half-life Products. • Factor XIII Assay	x 1.3ml paediatric citrate bottles (blue screw cap) for any combination of factor assays 	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Chromogenic Factor VIII Assay	2 x 1.3ml paediatric citrate bottles (blue screw cap) 	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Factor VIII and IX Inhibitor Assays	2 x 1.3ml paediatric citrate bottles (blue screw cap). This will also include the associated factor assay 	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. Haemolysed plasma is not suitable for analysis and should not be sent.

Test	Sample Requirements	Special Instructions
		• Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Investigations of von Willebrand Disease: • Factor VIII assay • von Willebrand Factor Antigen (vWF:Ag) • Ristocetin Cofactor Activity • vWF:CBA (collagen binding activity) if required.	2 x 1.3ml paediatric citrate bottles (blue screw cap) 	• Samples must arrive in the laboratory or be separated and frozen within 6 hours of collection. • Haemolysed plasma is not suitable for analysis and should not be sent. • Always notify the Laboratory if the test is urgent and discuss with one of the Haemophilia Medical staff
Platelet Function Analysis (PFA100)	By special arrangement only. Please contact the Laboratory to discuss sample requirements.	• **Performed only by agreement** with one of the Haemophilia Consultants and must be pre-booked with the Haemostasis and Thrombosis Laboratory. • Samples must be received in the Laboratory and tested within 4 hours of collection. • The samples **must** not be centrifuged
Platelet Aggregation	By special arrangement only. Please contact the Haemophilia Laboratory to discuss sample requirements	• **Performed only by agreement** with one of the Haemophilia consultants and must be pre-booked with the Haemostasis and Thrombosis Laboratory. • Samples must be received in the Laboratory and tested within 2 hours of collection.
Lupus Anticoagulant / Anti-phospholipid Screen (including anticardiolipin antibodies)	2 x 1.3ml paediatric citrate bottles (blue screw cap)  and 1 x 1.3ml plain serum sample (red screw cap) 	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection

Test	Sample Requirements	Special Instructions
Anticardiolipin Antibodies (Included in thrombophilia screen and Lupus Anticoagulant screen).	1 x 1.3ml plain serum sample (red screw cap) 	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection
Anti-Beta2-Glycoprotein 1 Antibodies	1 x 1.3ml plain serum sample (red screw cap) 	• Samples must be received in the laboratory or be separated and frozen within 6 hours of collection
Thrombophilia Screen: Including • Protein C • Protein S • Antithrombin • Lupus Anticoagulant • Anticardiolipin antibodies • Factor V Leiden G1691A • Prothrombin Gene G20210A	Testing is not generally advised in children under the age of sixteen years. In the first instance please discuss with one of the Haemophilia Consultants and then contact the Haemostasis and Thrombosis Laboratory for sample requirements	• Please discuss with one of the Haemophilia Consultants before collecting the samples. • As it can be difficult to collect sufficient samples from a child to perform a full thrombophilia screen, please clearly highlight on the request the most important assays for your patient.
Factor V Leiden G1691A Mutation (also included in thrombophilia screen)	Testing is not generally advised in children under the age of sixteen years. In the first instance please discuss with one of the Haemophilia Consultants and then contact the Haemostasis and Thrombosis Laboratory for sample requirements	• Please discuss with one of the Haemophilia Consultants before collecting the samples
Prothrombin Gene G20210A Mutation (also included in thrombophilia screen)	Testing is not generally advised in children under the age of sixteen years. In the first instance please discuss with one of the Haemophilia Consultants and then contact the Haemostasis Laboratory for sample requirements	• Please discuss with one of the Haemophilia Consultants before collecting the samples
Heparin Induced Thrombocytopenia Assay (HIT)	1 x 1.3ml plain serum sample (red screw cap) 	• Performed only by agreement with one of the Haemophilia Medical staff. • In all cases please contact the Haemostasis and Thrombosis Laboratory to discuss before sending the sample • A HIT clinical score is required for full interpretation of the results. This must be written on the accompanying request form, or in the clinical details for electronic requests.

25 REFERENCE RANGES

Test results are displayed with the appropriate reference ranges on both electronic and hardcopy reports, and results lying outside these range are highlighted in bold or flagged with an asterisk (*) to aid interpretation. Tests that do not have a numerical value are reported with interpretative comments dependant on the result.

A reference range for a laboratory test is a statistically-derived numerical range of results that is determined by testing a sample of “healthy” individuals. Reference ranges are conventionally set to give the range of values which would be found in approximately 95% of the “normal, healthy” population. This means that 5% (or 1 in 20) of the normal population will have a test result outside of the reference range.

Reference ranges can also be affected by a number of other factors including age, gender and pregnancy. Therefore, it is important to remember that reference ranges are provided for guidance in clinical decision making, rather than for prescriptive use, and the upper and lower limits of a reference range are not absolute and do not necessarily define “normal” and “abnormal”, but are points at which the probability of clinical significance tends to increase.

Our local reference ranges are shown in the table below and apply to East Kent Hospitals Trust only and may not be the same as in other hospitals/Laboratories. These ranges have been determined in a number of different ways.

Historically the reference ranges were established in-house by attending local blood donor sessions, collecting a minimum of 120 samples from carefully selected normal donors, analysing the samples and calculating the 95% confidence limits. However due to the cost, ethical issues of consent and practical logistics of this process, most of our reference ranges are now determined by performing a small verification exercise according to the Clinical and Laboratory Standards Institute (CLSI) Defining, Establishing and Verifying Reference Intervals Guidelines (EP28- A3C) to confirm that the original historic ranges are transferable to our current methodologies and instrumentation, or by confirming, again according to the CLSI Guidelines, that the manufacturer’s quoted reference ranges are applicable to the local population and analytical conditions. For some of our more esoteric tests the reference ranges are based on expert consensus in the literature or recommendations in national guidelines, and in a small number of cases solely on the manufacturer’s quoted reference range.

The source of the specific reference range for each test is indicated in the reference range table below according to the following key:

Reference Range Key:

- **HV** - Historic in-house reference range verified for current methodology/instrumentation according to CLSI Guidelines
- **MV** - Manufacturer’s quoted reference range verified for the local population and analytical conditions according to CLSI Guidelines.
- **IV** – Fully verified in-house range.
- **MR** - Manufacturer’s quoted reference range
- **CO** - Consensus expert opinion in the literature
- **NG** - National Guidelines

Test Name	Reference Range	Source of Reference Range
PT	12-16 seconds	HV
INR (only used for monitoring patients on oral anticoagulant therapy with vitamin K antagonists).	0.8 – 1.2 **Therapeutic Range:** 2.0 – 4.0 *(NB: The therapeutic range varies depending on the reason for anticoagulation and the patient's clinical history).*	HV NG
APTT	22-35 seconds	HV
Fibrinogen	**Adult Range** (≥16 years):1.9-4.3 g/L **Paediatric Ranges:** **0 to 31 days:** 1.92 – 4.01 g/L **1 month to 12 months:** 0.82 – 3.83 g/L **1 year to 4 years:** 1.62 – 4.01 g/L **5 years to 9 years:** 1.99 – 4.09 g/L **10 to 15 years:** 2.12 – 4.33 g/L	HV CO
Reptilase Time	14-19 seconds	HV
Thrombin time (TT)	13-20 seconds	HV
D-Dimer	0.05-0.5 ug/ml	MV
LMWH Anti-Xa	**Prophylactic Anticoagulation:** For effective prophylactic anticoagulation, anti-Xa levels should be in the range: 0.2 – 0.4 IU/ml. **Therapeutic Anticoagulation:** For effective therapeutic anticoagulation, anti-Xa levels should be in the range: 0.5 – 1.0 IU/ml.	CO
Rivaroxaban Anti-Xa	**Peak plasma concentrations:** 100 to 400 ng/ml **Trough plasma concentrations:** 20 to 150 ng/ml	CO
Apixaban Anti-Xa	**Peak plasma concentrations:** 62 to 128 ng/ml **Trough plasma concentrations:** 21 to 50 ng/ml	CO

Test Name	Reference Range	Source of Reference Range
Thromboelastography (TEG 5000)	• **R** (Reaction time): 2.5 – 7.5 minutes • **K** (Clot firmness): 0.8 – 2.8 • **α** (Kinetics of clot): 55.2 – 78.4 degrees • **MA** (Maximum amplitude): 50.6 – 69.4 mm • **CI** (Coagulation Index): -3 - +3 • **LY30** (% Lysis 30 min after MA): 0 – 8 % Plus interpreted as normal or abnormal based on visual assessment of the TEG traces by a trained eye	MR
Antithrombin Activity	80-132 %	MV
Antithrombin Immunogenic	80-120 %	MV
Protein C Activity	70-150 %	MV
Free Protein S	Males: 70-148 % Females: 50-134 %	MV
Lupus Anticoagulant Assays	**Dilute Russell Viper Venom Time Assays (dRVVT)** • dRVVT Screen: 0.80 – 1.20 ratio • dRVVT Screen 50:50 Mix: 0.88 – 1.09 ratio • dRVVT Confirm: 0.90 – 1.10 ratio • dRVVT Confirm 50:50 Mix: 0.91 – 1.03 ratio • dRVVT % Correction: -15.9 – 11.6 % **Lupus Sensitive APTT Assays (APTT-LA)** • APTT-LA Screen: 0.84 to 1.16 ratio • APTT-LA Screen 50:50 Mix: 0.91 to 1.07 ratio • APTT-LA Confirm: 0.89 to 1.11 ratio • APTT-LA Confirm 50:50 Mix: 0.94 to 1.05 ratio • APTT-LA % Correction: -9.7 to 9.5 % An overall interpretation of Lupus Anticoagulant negative or positive is also provided based on the dRVVT and APTT-LA results.	IV (all Lupus Anticoagulant assays)

Test Name	Reference Range	Source of Reference Range
vWF:AG	50-160 %	MV
vWF:RICOF	50 – 200 iu/dl	MV
vWF:CBA (Collagen Binding Activity)	50 – 130 iu/dl	IV
Factor XIII Assay	74-141 iu/dl	MR
Factor XII Assay	50-200 iu/dl (New born 50% lower)	MV
Factor XI Assay	70-160 iu/dl (New born 30-50% lower)	IV
Factor IX Assay	60- 150 iu/dl	MV
Factor IX Assay for extended half-life products.	60- 150 iu/dl	MV
Factor VIII Assay (Clotting Based Test)	50-200 iu/dl	MV
Chromogenic Factor VIII Assay	50-200 iu/dl	MV
Refacto Factor VIII Assay	50-200 iu/dl	HV
Factor X Assay	50-200 iu/dl (New born 30-50% lower)	HV
Factor VII Assay	50-200 iu/dl	HV
Factor V Assay	50-200 iu/dl	HV
Factor II Assay	50-200 iu/dl	HV
Bethesda Assay For Factor VIII or Factor IX Inhibitors	• No Inhibitor detected: Reported as negative. • Inhibitor present: Reported as the Bethesda Titre (Bu/ml).	N/A (inhibitor either not detected or detected)
Anticardiolipin Antibodies (ACA) IgM and IgG	**IgG ACA** • <10 GPL: Negative • 10-20 GPL: Weak positive • 20-80 GPL: Moderate positive • >80 GPL: Strong positive **IgM ACA** • <7 GPL: Negative • 7-20 MPL: Weak positive • 20-80 MPL: Moderate positive • >80 MPL: Strong positive **NB: All ACA results are reported as positive or negative on the basis of the numerical results above**	MR (same for both ACA assays)

Test Name	Reference Range	Source of Reference Range
Anti-Beta-2-Glycoprotein 1 Antibodies	• <5 U/ml: Negative • 5-10 U/ml: Borderline Positive • >10 U/ml: Positive **NB: Reported as positive or negative on the basis of the numerical results.**	MR
Platelet Function Analysis (PFA)	• **Collagen/Epinephrine:** Closure Time: 85 – 165 seconds • **Collagen/ADP:** Closure Time: 71 – 118 seconds	HV
Platelet Aggregation	>50% aggregation is considered to be normal. Reported as normal or abnormal based on visual assessment of each aggregation response compared to a normal control sample analysed at the same time.	NG (verified locally)
Heparin Induced Thrombocytopenia Assay (HIT)	• Mean OD value **< 0.40:** Negative • Mean OD value **≥ 0.40:** Positive	MR
Factor V Leiden G1691A mutation	Depends on whether the mutation is not detected or detected in the heterozygous or homozygous state as below. • FV Leiden G1691A mutation not detected: **Negative.** • FV Leiden G1691A mutation detected in the heterozygous state: **Heterozygous Positive.** • FV Leiden G1691A mutation detected in the homozygous state: **Homozygous positive.**	N/A (genetic mutation is either not detected or present in the heterozygous or homozygous state)
Prothrombin Gene G20210A Mutation	Depends on whether the mutation is not detected or detected in the heterozygous or homozygous state as below. • Prothrombin Gene G20210A mutation not detected: **Negative.** • Prothrombin Gene G20210A mutation detected in the heterozygous state: **Heterozygous Positive.** • Prothrombin Gene G20210A mutation detected in the homozygous state: **Homozygous positive.**	N/A (genetic mutation is either not detected or present in the heterozygous or homozygous state)

Our reference ranges are subject to regular review and may change from time to time due to changes in or updating of the methodology/instrumentation used in the Laboratory. Any changes to the reference ranges will be clearly highlighted with a comment on the final report for a period of six months following the change.

For further information on our reference ranges or for advice on the interpretation of specific patient results, please telephone the Haemostasis and Thrombosis Laboratory and ask to speak to a senior member of staff.

26 TURNAROUND TIMES

Test	Turnaround Time	Test	Turnaround Time
Routine Coagulation Tests • Coagulation screen (PT and APTT) • INR for monitoring oral anticoagulant therapy • APTT ratio (APTTr) for monitoring heparin therapy • D-Dimer • Thrombin time • Fibrinogen • Reptilase time	**1 hour** for all samples from ECC, CDU, ITU and DVT Clinic and any other samples marked as urgent **4 hours** for all other routine coagulation samples	**Thrombophilia Screen:** • Protein C • Protein S • Antithrombin • Lupus Anticoagulant • Anticardiolipin antibodies • Factor V Leiden G1691A mutation • Prothrombin Gene G20210A	**14 working days for the full screen or any individual component of a thrombophilia screen.**
Factor Assays: • One stage factor assays (factors II, V, VII, VIII, IX, X, XI, and XII) • Chromogenic Factor VIII Assay • Refacto Factor VIII Assay • Factor IX Assay for Extended Half-life Products. • Factor XIII Assay	**7 working days or 4 hours if clinically urgent** (and discussed and agreed with Haemophilia consultant)	**Anticardiolipin Antibodies** (also included in thrombophilia screen and Lupus Anticoagulant screen).	**14 working days**
von Willebrand Investigations: • von Willebrand Factor Antigen (vWF:Ag) • Ristocetin Cofactor Activity • Factor VIII (clotting based)	**7 working days or same day if clinically urgent** (and agreed with Haemophilia consultant)	**LMWH Anti-Xa Assay**	**2 working days or same day if clinically urgent**

Test	Turnaround Time	Test	Turnaround Time
Factor VIII and IX Inhibitor Assays	**7 working days or 4 hours if clinically urgent** (and discussed and agreed with Haemophilia consultant)	**Rivaroxaban Anti-Xa Assay** **Apixaban Anti-Xa Assay**	**7 working days or same day if clinically urgent**)
Factor V Leiden G1691A Mutation (also included in thrombophilia screen)	**14 working days**	**Prothrombin Gene G20210A Mutation** (also included in thrombophilia screen)	**14 working days**
Lupus Anticoagulant / Anti-phospholipid Screen (including anticardiolipin antibodies)	**14 working days**	**Anti-Beta2-Glycoprotein 1 Antibodies**	**14 working days**
Heparin Induced Thrombocytopenia Assay (HIT)	**1 working day**	**PFA-100** **Platelet Aggregation Studies**	**1 working day** **7 working days**
Whole Blood Coagulation by Thromboelastography (TEG)	**1 working day**		