

# HISTOCOMPATIBILITY LABORATORIES

## SERVICE PROVISION USER GUIDE

saving the lives of people with blood cancer

[anthohnolan.org](http://anthohnolan.org)

**ANTHONY  
NOLAN**

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## 1. INTRODUCTION

Here at Anthony Nolan, we save the lives of people with blood cancer. We use our register to match incredible individuals willing to donate their stem cells to people in desperate need of transplants. We conduct pioneering research into the treatment of bone marrow disorders and look for new ways to improve the effectiveness of stem cell transplants.

The Anthony Nolan Histocompatibility Laboratories provides Histocompatibility and Immunogenetics (H&I) services to donors and patients awaiting Haematopoietic Stem Cell (HSC) transplants and to the renal transplant patients of the Royal Free Hospital. In addition, the laboratories provide Human Leukocyte Antigen (HLA) related disease association and drug-resistance testing.

This prospectus is aimed at the following individuals and organisations to ensure that all those affected by the services provided by Anthony Nolan Histocompatibility Laboratories are informed of the processes undertaken:

- Transplant centres and H&I staff in the UK and overseas
- General practitioners
- Patients in need of a HSC transplant and their families in the UK and overseas
- Potential HSC donors
- Government or Professional agencies
- The wider national and international scientific and medical community
- Anthony Nolan supporters and fundraisers
- International registries
- Hospital haematology units

## 2. ANTHONY NOLAN DEPARTMENTS

**Our organisation is made up of several departments, all working together to ensure we save as many lives as we can.**

This brochure outlines the services specifically offered by the **Anthony Nolan Histocompatibility Laboratories**.

Our other departments also have an important role to play in achieving our aim, such as Operations and Patient Services, Scientific Research, Information Technology, Cord, Finance and Resources and Engagement. For further details of these departments please visit <http://www.anthonynolan.org>.

We act as the UK's 'hub' for volunteer unrelated HSC transplantation.

As such, Anthony Nolan co-ordinates all aspects of extending a donor search internationally, from foreign donor and cord blood sample requests through to donor work-up for donation and import of haematopoietic stem cell products.

For a comprehensive overview of the services we provide, look at the Anthony Nolan Operations Service User Guide. (<http://www.anthonynolan.org/Healthcare-professionals/Services/Operations-user-guide.aspx>)

The Anthony Nolan Histocompatibility Laboratories are accredited by United Kingdom Accreditation Service (UKAS) to ISO15189:2012, the European Federation for Immunogenetics (EFI) and regulated by the Care Quality Commission (CQC). The certificates can be viewed on our website <http://www.anthonynolan.org/about-us/accreditation-and-regulation>.

### a. DATA PROTECTION

Anthony Nolan shall comply with the Data Protection Act 2018 and the EU General Data Protection Regulation 2016/679.

Please refer to our [privacy policy \(www.anthonynolan.org/privacy-policy\)](http://www.anthonynolan.org/privacy-policy) for further information on how Anthony Nolan uses and stores personal information.

## 3. SERVICES SUPPORTING HSCT

Anthony Nolan H & I services, headed by a Consultant Clinical Scientist, have been supporting haematopoietic stem cell transplantation for over 30 years. Our dedicated Laboratory situated on our Royal Free Hospital site is equipped to deliver a world leading service for your patients, delivering cutting edge HLA typing and research-led advice on the best donor options. As the largest H&I laboratory within the UK, we use our knowledge and experience to support 25% of all UK based allogeneic transplants. We have the capacity to

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flex to your needs, and together with our registry services we can offer a seamless end to end service for all your patients.

### **a. REGISTRY SERVICES**

To date Anthony Nolan has facilitated over 16,000 transplants since the first unrelated donor transplant was performed in 1979. Our ability to adapt and evolve with the techniques and technologies over the years has ensured we are able to continue offering excellence in our service and qualified expert advice well received by our all customers.

The Operations and Patient Services (OPS) division is responsible for overseeing the donation process once a donor has been identified as a suitable match. The service, tailored to suit your needs, will be delivered by our dedicated and fully committed staff to your specification and within regulatory requirements.

The Histocompatibility Laboratory works in conjunction with OPS by HLA typing people joining the register and typing of mothers and their cord blood to support the cord bank. It also provides extended typing requests for HLA, virology screening (CMV, Hepatitis B, Hepatitis C and HIV) for donor selection, ABO blood grouping and CCR5 delta 32. Requests for these services can be made via your UK search coordinator or your national registry hub.

### **b. HLA TYPING**

Anthony Nolan uses pioneering techniques to provide allelic level typing at HLA- A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 ensuring you are able to make the right decision for your patient. HLA typing is performed on all donor and patient samples utilising our Third Generation Sequencing technique. This uses Pacific Biosciences Single Molecule Real Time sequencing to generate full gene, phased, unambiguous HLA types reported to you in 7 days. To ensure we are able to deliver to your timeframes and clinical requirements a comprehensive range of techniques will be utilised should you require an urgent service. Additional loci such as HLA-DQA1, DPA1 and CCR5 can be typed if appropriate. All patients and their selected donors should be HLA typed twice prior to transplantation. Request forms are available by contacting [clinicalservices@anthohnolan.org](mailto:clinicalservices@anthohnolan.org) or are downloadable from [www.anthohnolan.org](http://www.anthohnolan.org).

### **c. ANTIBODY ASSESSMENT**

Patients being considered for a mismatched transplant should be screened for HLA antibodies. To perform these tests Anthony Nolan utilises Luminex XMAP analysers for a preliminary screen followed by a single antigen assessment if required. In certain circumstances a crossmatch may be required to assist in final donor choice. Should HLA antibodies be present in

the patient against the selected donor (donor specific antibodies or DSA), you may decide to perform antibody removal. In this case antibody monitoring can be performed to assess the DSA levels to facilitate transplantation.

#### **d. ADDITIONAL LABORATORY SERVICES**

The Anthony Nolan Laboratory is accredited to perform ABO, Rh blood grouping and screening for exposure to relevant viruses. This includes HIV, HepB, HepC and CMV. Where positivity to HepB, HepC and HIV is detected a repeat test is carried out through a referral Laboratory.

#### **e. GRAFT IDENTIFICATION ADVISORY SERVICE (GIAS)**

The Graft Identification Advisory Service is supported by a panel of clinical experts to ensure the best donor options are pursued for your patient. Should an unrelated donor or cord blood unit be required for your patient our qualified Search and Selection team will search the UK register and where applicable overseas registries. The team will recommend unrelated donors based on the pre-agreed selection criteria for your patient and initiate the procurement and shipment of blood samples for verification typing carried out at our Laboratory. For each donor, both related and unrelated, comments on the matching suitability will be provided based on our research supported criteria. Our expert team are available to discuss recommendations and further testing strategies with you to ensure the optimal outcome for your patient.

#### **f. CHIMERISM**

Anthony Nolan works with a partner referral Laboratory to provide post-transplant Chimerism monitoring. Lineage specific analysis can also be performed. Please contact the laboratory for further details at [clinicalservices@anthohnolan.org](mailto:clinicalservices@anthohnolan.org)

#### **g. TARGET TURNAROUND TIMES**

We aim to process and report at least 75% of patients, potential related and unrelated donor samples within seven working days. If you require urgent typing please contact [clinicalservices@anthohnolan.org](mailto:clinicalservices@anthohnolan.org) to discuss time frames.

## 4. SERVICES SUPPORTING SOLID ORGAN TRANSPLANTATION

The Histocompatibility Laboratories Solid Organ team, provides a service to support the requirements of the renal transplant unit of the Royal Free Hospital, London. This service is funded by the Royal Free London NHS Foundation Trust.

### a. RENAL TRANSPLANTATION

For kidney transplantation, potential recipients are HLA typed by DNA based methods at HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQB1 and DPB1. Two independent samples are tested for each patient prior to registration with Organ Donation and Transplantation (ODT), part of National Health Service Blood and Transplant, for the Transplant List. Patients are also screened for HLA alloantibodies and if present, the specificity of such antibodies is defined. Patients are screened at three monthly intervals in accordance with British Transplantation Society standards ([www.bts.org.uk](http://www.bts.org.uk)). Family members may also be HLA typed to resolve patient's HLA haplotypes and to aid definition of antibodies arising from sensitisation through pregnancy.

#### i. CADAVERIC TRANSPLANTATION

A 24-hour, seven days a week, on-call service is available to provide tissue-typing and cross-matching facilities on an on-call basis. The London team Transplant Coordinators (NTTC) also known as Specialist nurses for organ donation (SNOD), inform laboratory staff of potential cadaver organ donors in 'local' hospitals and arrange for EDTA blood to be collected and sent to the laboratory for HLA typing. All "local" cadaver donors are HLA typed by serology (HLA-A, -B, -DR, -DQ) and by DNA methods (HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQB1, -DPB1). ODT is notified of donor HLA type by email. Non-local donors also have their HLA type confirmed by serology during on-call hours and subsequently by DNA methods.

Crossmatching between donor and potential recipient is performed using Flow Cytometry methods. This enables detection of the presence of donor specific antibodies in the patient serum, which if present are a contraindication for transplantation.

A virtual crossmatch may be performed on un-sensitised patients. This is when the predicted result is negative, and the transplant can proceed before the crossmatch is completed. All results are reported to on duty physicians and surgeons.

#### ii. LIVING DONOR TRANSPLANTATION

Where a living related, or unrelated donor transplant is being considered, all potential donors are blood group typed to determine compatibility. HLA typing (HLA-A, -B, -C, -DRB1/3/4/5 and -DQB1) is performed to determine compatibility. Virtual cross matching is performed between patient and potential donors to predict a positive or negative result at the initial time of donor selection. A Flow Cytometry crossmatch is performed with the selected donor within 7-14 days of the proposed transplant. All results are communicated in writing to the transplant nurse and clinicians. Serum samples from all recipients of a transplant are received and screened at intervals in accordance to British Transplant Society guidelines.

### b. TARGET TURNAROUND TIMES

Service	TAT
New patient on National waiting list	10 days
Cadaver renal crossmatch	7 hours
Local donor offers HLA type (From blood draw to reporting HLA type)	8 hours
Live virtual renal donor crossmatch (Flow Cytometry) + HLA and Blood group	7 days
Final Live donor crossmatch (Flow cytometry)	48 hours
Routine HLA antibody screening (by Luminex)	10 days

## 5. DISEASE ASSOCIATION

HLA typing by DNA methodology will be undertaken to provide support in the diagnosis of associated diseases and drug hypersensitivity reactions, including but not limited to:

Disease	Associated HLA
Ankylosing Spondylitis	HLA-B*27
Narcolepsy	HLA-DRB1*15 and -DQB1*06
Bechet's Disease	HLA-B*51
Coeliac Disease	HLA-DQ2 and -DQ8
Abacavir Hypersensitivity	HLA-B*57:01

For more details on disease association tests and costs incurred please contact the laboratory: [clinicalservices@anthonymolan.org](mailto:clinicalservices@anthonymolan.org)

### a. TARGET TURNAROUND TIME

Our target turnaround time for reporting disease association requests is for 75% of reports to be sent within five working days.

### b. HAEMOCHROMATOSIS MUTATION DETECTION

Haemochromatosis is an autosomal recessive disorder which causes an increase in iron absorption leading to iron overload. Two mutations within the HFE gene (found telomeric to the HLA genes on chromosome 6) have been found to be commonly associated with the disorder. These mutations which alter amino-acid 63 (histidine to aspartate) and amino-acid 282 (cysteine to tyrosine) are detected by the PCR-SSP technique. Two additional mutations are also tested: one is a mutation that alters amino acid 65 (Serine to Cysteine) and the other is a splice site mutation (1VS3+1G/T). Please contact the laboratory for a request form. [clinicalservices@anthonymolan.org](mailto:clinicalservices@anthonymolan.org)

## 6. CONTRACT HLA TYPING

The Histocompatibility Laboratories can undertake low and high throughput contract typing for examples supporting academic research studies, or clinical trials. For more details contact the laboratory: [typingservices@anthonymolan.org](mailto:typingservices@anthonymolan.org).

## 7. HLA TYPING – AN INTRODUCTION

HLA typing is performed to define compatibility between donor and patient. An individual's HLA type is determined by a group of genes which produce proteins expressed on virtually all tissues in the human body. These genes are extremely variable (or polymorphic), making it unlikely that any two random individuals would have an identical HLA type. There are different laboratory methods which are utilised to determine HLA type, and these methods can be divided into two groups. The first is based on the serological recognition of the HLA protein at the cell surface. This test utilises many different antibodies with specificity against different HLA molecules to determine HLA type. The second group of methods work at the DNA level directly and attempt to identify the genetic make-up of the HLA genes. The HLA system has many different polymorphisms in the population. There are currently over 10000 different HLA alleles (or variants) (<http://hla.alleles.org>), therefore the typing methods and interpretation of results can appear complex. A full list of HLA alleles currently defined by the WHO Nomenclature Committee for Factors of the HLA System can be found at: <http://www.ebi.ac.uk/imgt/hla/>

**a. THIRD GENERATION SEQUENCING (TGS)**

TGS utilises the Pacific Biosciences Single Molecule Real Time (SMRT) sequencing system. The ability of SMRT sequencing to generate sequence data on long amplicons means it is possible to sequence an entire HLA gene in isolation producing ultra-high-resolution HLA type.

**b. DNA BASED TYPING PCR-SEQUENCING-BASED TYPING (SBT) TESTING**

Direct sequencing of PCR products is performed on an automated DNA sequencer using dye terminator chemistry. The Laboratory utilises both an in house developed methodology and the commercially available SECORE HLA typing kit.

**c. DNA BASED TYPING: SEQUENCE SPECIFIC PRIMER (SSP) TESTING**

SSP typing utilises a panel of PCR primer pairs which target known polymorphic nucleotide motifs within HLA alleles. The presence or absence of a PCR product (in the presence of a positive internal control PCR product) determines the presence or absence of a particular nucleotide sequence this pattern is interpreted to give the HLA type. This technique is used when a HLA type is required quickly.

**d. DNA BASED TYPING TECHNIQUES: SEQUENCE SPECIFIC OLIGONUCLEOTIDE PROBE (SSOP) TESTING**

The SSOP assay involves the incubation of locus specific polymerase chain reaction (PCR) products (e.g. HLA-A) with a panel of immobilised oligonucleotide probes with sequences complementary to polymorphic sequences within the HLA alleles. We are currently using Luminex® xMAP® in which the oligonucleotide probes are immobilised on polystyrene microspheres (or beads). Reactivity between PCR product and beads is detected by the measurement of fluorescence at two wavelengths, the first to identify the specificity of the bead and the second to determine if PCR product has bound.

**e. SEROLOGICAL TYPING**

HLA typing can be performed by complement dependent lymphocytotoxicity reaction (serology). Viable peripheral blood mononuclear cells are required for this assay. CD8+ T-cells and/or CD19+ B-cells are purified from whole blood and incubated against a panel of antibodies with specificity against polymorphic epitopes expressed on HLA-A, -B proteins (CD8+ T-cells) or HLA-DR and -DQ proteins (CD19+ B-cells). In the presence of complement cells expressing HLA, proteins which react with a particular antibody are

lysed, allowing these damaged cells to uptake a stain which is detected by fluorescent microscopy. The pattern of negative and positive reactions is scored and interpreted to give a serological HLA type.

Serological HLA typing is the quickest method to establish identity within a family, and it also allows the identification of null alleles.

#### **f. VIROLOGY AND BLOOD GROUP TYPING**

Human sera are screened for the presence of CMV (antibody), HIV1/2 (antibody/antigen), hepatitis B (antigen) and C (antibody) infection by ELISA. Any ambiguous/equivocal results may be confirmed by referral to the CPA accredited Department of Virology at Royal Free Hospital, Pond Street, London, NW3 2QG

Blood group typing is performed by haemagglutination reaction to detect red blood cell antigens and determine ABO and Rh(D) blood group. Any ambiguous/equivocal results may be confirmed by referral to the CPA accredited Blood Transfusion team at Royal Free Hospital, Pond Street, London NW3 2QG.

#### **g. HLA ANTIBODY SCREENING**

Luminex technology is used to establish the presence and specificity of HLA class I and II reactive antibodies in both pre- and post-transplant patients.

Luminex® technology uses fluorescent Micro beads coated with purified HLA class I and II proteins. The beads are incubated with patient sera and bound antibodies detected after reaction with a fluorescently labelled IgG specific secondary antibody. Test sera are screened to initially determine the presence of HLA class I or II specific antibodies. Sera scoring positive for HLA specific antibodies are further analysed with Luminex kits designed to define HLA specificities. Highly sensitised patients with IgG antibodies reactive with multiple HLA class I or II antigens are further characterised with Luminex beads possessing immobilised recombinant single class I or II molecules. This provides the highest resolution of HLA-specific antibody analysis. Currently the laboratory uses Luminex microbead products from two different manufacturers.

#### **h. CROSSMATCHING**

The purpose of the crossmatch (XM) test is to determine the presence of pre-formed antibodies in a patient that are reactive with HLA antigens on donor cells. This could cause hyper acute rejection of a renal graft. Flow-cytometric (FC) tests are performed by reacting both fresh and selected historic recipient sera with separated donor T and B-cells (allogeneic XM), and recipient T and B-cells (autologous XM). The results of these tests, in conjunction with the

known immunological and clinical parameters of a potential renal transplant recipient, are used to advise renal transplant surgeons on the likely risk (high, intermediate or low) of graft rejection occurring.

A virtual crossmatch may be performed if the patient has a consistently negative antibody screening history. This means that the transplant can proceed before the crossmatch results are available.

HLA antibody screening: tests are reported as positive or negative. HLA antibody specificity identification: antibody specificities identified at a value higher than our validated positive cut off are reported in the context of any previous result, a patient sensitisation history and other relevant clinical information.

The pre-transplant immunological risk assessment is made on the allogeneic crossmatch result, the crossmatch method, whether a positive result was obtained for a historical or current serum and the antibody screening result. The interpretation of these results is made according to the [BTS/BSHI guidelines](#).

#### **i. PARTICIPATION IN EXTERNAL QUALITY ASSURANCE SCHEMES**

The Histocompatibility Laboratories take part external quality assurance programmes covering the scope of the service. Further details are available upon request.

#### **j. REFERRAL LABORATORIES**

To support the HLA typing for the addition of new donors to the Anthony Nolan register, at times of maximum capacity, HLA typing may be outsourced to a partner Laboratory with current EFI, ASHI, ISO or other relevant accreditation.

## 8. HISTOCOMPATIBILITY LABORATORIES INFORMATION

### a. REQUEST FORMS AND SAMPLE LABELLING

All samples sent to the laboratory MUST be accompanied by a fully completed, appropriate request form.

Request forms are available for the following tests either [online](#) or by contacting the [laboratory](#):

Patient and donor histocompatibility testing.

Disease association studies

Haemochromatosis mutation detection

Cord Verification Typing

Renal transplant histocompatibility testing (patient and/or donor tests)

Please ensure you are using the most current version of the request form.

Please write **clearly in black ink and use block capitals** when completing request forms. **All fields must be fully completed.** All details must be checked carefully to ensure they are correct

If a sample is clinically urgent then this must be marked on the request form. Please restrict the use of urgent requests to those that are absolutely necessary.

Samples must be correctly labelled with **at least three of the following identifiers:**

Full name (First and Surname)

Date of Birth

Hospital Number (if available)

NHS/CHI number

In addition, samples MUST be labelled with:

Date & time of bleed

Requesting Location (Hospital/TC name)

Incorrectly or insufficiently labelled tubes or request form will result in testing being delayed, or the sample discarded and the patient or donor having to be re-bled.

It is the requesting centre's responsibility to ensure appropriate patient and donor consent as stipulated by the Human Tissue Act 2004 is obtained for the requested tests.

#### **b. SAMPLE TYPE REQUIREMENTS**

**Sample types are shown in the table below and indicated on the request form.**

<b>Test</b>	<b>Sample Required</b>
HLA typing for HSCT (patient and donor)	2 x 4ml EDTA
Disease Associations	1 x 4ml EDTA
Virology screening	1 x 4ml clotted
Blood grouping	1 x 4ml EDTA
New renal patient	2 X 6mlml EDTA + 2x 6ml clotted
Renal crossmatch	7 x 6ml EDTA (donor) or spleen or lymph node as appropriate and 1 x 2 x 6ml clotted + 7 x 6ml EDTA (patient)
Renal antibody screening	1 x6ml EDTA
Renal donor	7 x 6ml EDTA

All samples should be bled by an appropriately qualified phlebotomist or medical practitioner in accordance with local procedures.

All samples should be stored and shipped at room temperature. Please ensure all mailing containers are compliant with UN3373 and IATA 650 transport regulations. All pathological specimens should be packaged by a recognised laboratory or institution, a qualified medical practitioner or a dental practitioner.

#### **c. FACTORS KNOWN TO AFFECT PERFORMANCE OF TESTS**

In general samples should be sent to the laboratory without delay at ambient temperature as certain tests require viable cell populations. Furthermore, our ability to extract quality DNA can be reduced in old samples.

It is important to collect samples into the correct tubes. Please ensure the correct anticoagulant (usually EDTA) or no anticoagulant (clot) is used

(see table in section 8b). It is also important to supply adequate volumes of blood to allow completion of testing.

Certain tests can be affected if a patient has a low white blood cell count as a result of drug treatment or disease. Please indicate on the request form if the patient is known to be leucopenic.

Serum samples for HLA antibody screening and cross matching must be received in the laboratory within 48 hours of being drawn. Serum must be used in downstream procedures within 48 hours of being drawn or stored at  $-70^{\circ}\text{C}$  until required.

For Flow cross matching; EDTA blood used for lymphocyte isolation must be despatched at ambient temperature and reach the laboratory for processing within 48 hours of being drawn.

#### **d. HISTOCOMPATIBILITY LABORATORY ADDRESS AND OPENING HOURS -SECTION**

Postal address of Histocompatibility Laboratories:

Anthony Nolan Round Table Laboratories  
Royal Free Hospital  
Pond Street  
London  
NW3 2QG

The laboratories are open Monday to Friday, excluding bank holidays from 8am to 5pm. We also provide an out of hours service in support for solid organ transplantation at the Royal Free hospital.

**e. TIME LIMITS FOR REQUESTING ADDITIONAL EXAMINATIONS**

Samples from all patients who have undergone a transplant and their donors (related and unrelated) are securely stored frozen in the laboratories as either DNA or whole blood in accordance with national guidelines.

Serum samples from renal transplant patients (pre and post-transplant) are securely stored frozen in the laboratories as sera in accordance with national guidelines.

**f. HISTOCOMPATIBILITY LABORATORIES CONTACT DETAILS**

Laboratory reception telephone number: 020 7284 8348

Laboratory email address

(general enquiries): [Laboratories@anthohnolan.org](mailto:Laboratories@anthohnolan.org)

Clinical / Result Enquiries:

[clinicalservices@anthohnolan.org](mailto:clinicalservices@anthohnolan.org)

Commercial HLA typing enquiries:

[typingservices@anthohnolan.org](mailto:typingservices@anthohnolan.org)

## 9. HISTOCOMPATIBILITY LABORATORIES DEPARTMENT SENIOR STAFF

### **Clinical Director of Laboratories Consultant Clinical Scientist**

Dr. Katy Latham BSc, PhD, HCPC registered, FRCPath

### **Co-Director of Laboratories**

Professor Steven G E Marsh  
BSc PhD ARCS

### **Scientific Director**

Professor Alejandro Madrigal  
MD, PhD, FRCP, FRCPath, DSc

### **Consultant Clinical Scientist**

Henry Stephens, BSc, PhD, HCPC registered

### **Head of Quality (Quality Manager)**

Dr Emma Radway-Bright, BSc, PhD

### **Medical Director**

Dr Chloe Anthias, MRCP

## 10. COMPLAINTS PROCEDURE

Comments on the service provided by the Laboratory are welcome and help us to improve. Please send details in an email to the Quality Team: [QualityTeam@anthohnolan.org](mailto:QualityTeam@anthohnolan.org)

If you wish to make a formal complaint regarding the services provided by the Anthony Nolan Histocompatibility Laboratories, or to report a Serious Adverse Event or Reaction (SAE/SAR), please send details in an email to the Quality Team: [QualityTeam@anthohnolan.org](mailto:QualityTeam@anthohnolan.org)