# Histocompatibility Laboratories

## Service Provision User Guide

Version 12 | April 2023

ANTHONY NOLAN

saving the lives of people with blood cancer

# 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 Haematopoetic 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 **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.
**anthonynolan.org/clinicians-and-researchers/transplant-and-laboratory-services/transplantation-services/guides-and-forms**

The Anthony Nolan Histocompatibility Laboratories are accredited by United Kingdom Accreditation Service (UKAS) to ISO15189:2012 and the European Federation for Immunogenetics (EFI). The certificates can be viewed on our website: **anthonynolan.org/what-we-do/our-organisation/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 (**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 Laboratory Director with the support of highly experienced Clinical Scientists, have been supporting haematopoietic stem cell transplantation for over 40 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 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 22,000 transplants since the first unrelated donor transplant was performed in 1973. 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 is a part of OPS and performs the HLA typing of 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 screening for the CCR5 delta 32 mutation. 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 high resolution to allelic level typing at HLA- A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 ensuring you can make the right decision for your patient. HLA typing is performed on all donor and patient samples utilising the Next Generation Sequencing (NGS) technique. NGS uses GenDx NGSGo to deliver excellent quality high resolution results, reported to you in a quick

turnaround. To ensure we can deliver to your timeframes and clinical requirements, a selection of techniques will be utilised should you require an urgent service. Additional results will also be reported for HLA-DQA1 and DPA1 wherever possible. All patients and their selected donors should be HLA typed twice prior to transplantation. Request forms are available by contacting **clinicalservices@anthonynolan.org** or are downloadable from **anthonynolan.org/clinicians-and-researchers/transplant-and-laboratory-services/transplantation-services/guides-and-forms**

### 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@anthonynolan.org**

### G. Target Turnaround Times

We aim to process and report at least 90% of patients, potential related and unrelated donor samples within seven working days. If you require urgent typing please contact **clinicalservices@anthonynolan.org** to discuss time frames.

Any significant delays to the service, including to the reporting of testing results will be communicated to our users.

# 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). 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<br>(From blood received in Labs to reporting HLA type) | 4 hours |
| Live virtual renal donor crossmatch<br>(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 |
| Ankylosing Spondylitis | HLA-B*27 |
| Narcolepsy | HLA-DRB1*15 and<br>-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@anthonynolan.org**

### A. Target Turnaround Time

Our target turnaround time for reporting disease association requests is for 90% of reports to be sent within seven 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 (IVS3+1G/T).

Please visit **anthonynolan.org/clinicians-and-researchers/services-transplant-centres/full-list-forms** to obtain a request form or contact the laboratory at **clinicalservices@anthonynolan.org**

# 6. Contract HLA Typing

The Histocompatibility Laboratories can undertake low and high throughput contract typing for example; supporting academic research studies, or clinical trials. For more details contact the laboratory: **typingservices@anthonynolan.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 These consist of a group of methods that 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 35,000 different HLA alleles (or variants) (**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: **ebi.ac.uk/imgt/hla**

### A. Next Generation Sequencing (NGS)

NGS utilises the GenDx NGSGo kit in conjunction with Illumina Sequencers. This technique can produce reliable high resolution to allelic level HLA results, testing for 11 possible HLA genes in one run. The well established short read technology is highly efficient and allows for all HLA testing to be performed in one pipeline.

### B. 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, in particular, when an HLA type is required quickly.

### C. 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 UKAS 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 UKAS accredited Blood Transfusion team at Royal Free Hospital, Pond Street, London NW3 2QG.

## D. 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.

## E. 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 of 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.

## A. Participation in Quality Assurance Schemes

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

**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.

| TEST | SAMPLE REQUIRED |
|---|---|
| HLA typing for HSCT (patient and donor) | 2 x 4ml EDTA or<br>2 x Buccal Swabs |
| 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 +<br>2 x 6ml clotted |
| Renal crossmatch | 7 x 6ml EDTA (donor) or spleen or lymph node as appropriate |
| Renal antibody screening | 1 x 6ml 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 leucopoenic.

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 -700°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.

Users of our services will be notified if there is any deviation from our accredited services.

## D. 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.

## E. Histocompatibility Laboratory Address, Opening Hours and Contact Details

Postal address of Histocompatibility Laboratories:

Anthony Nolan Laboratories
77B Fleet Road
London NW3 2QU

The laboratories core opening hours are Monday to Friday, excluding bank holidays from 9am to 5pm.

Clinical and scientific advice regarding testing and on the interpretation of results is available during these hours. See below for contact information.

We also provide an out of hours service in support for solid organ transplantation at the Royal Free hospital, 24 hours a day, 365 days a year. A Consultant clinical scientist is also available to medical staff for transplantation advice outside of the core working hours. See below for contact information.

Laboratory reception telephone number: 020 7284 8348

Laboratory email address
(General enquiries): **Laboratories@anthonynolan.org**

**Clinical advice and Result Enquiries relating to HSCT**
Email: **clinicalservices@anthonynolan.org**

Telephone contacts:
Clinical Services: 0207 2848346
Clinical Services: 0207 2848329

**H & I Clinical Consultant Lead/Principal clinical Scientist:**
0207 4246573 (office)
07884650581

**Commercial HLA typing enquiries:**
Email: **typingservices@anthonynolan.org**

**Clinical advice and Result Enquiries relating to Solid organ transplant (Royal Free Hospital)**
Email: **rftissuetyping@nhs.net**

Telephone contacts:
02077940500 Ext 35123
02072848341
07786278788 (Out of hours contact number)

Consultant Clinical Scientist: 07902915442

## 9. Histocompatibility Laboratories Department Senior Staff

**Director of Laboratory Operations**
Lisa Walsh

**Co-Director of Laboratories**
Professor Steven G E Marsh BSc PhD ARCS

**Chief Medical & Scientific Advisor**
Antonio Pagliuca

**Consultant Clinical Scientist**
Henry Stephens, BSc, PhD, HCPC registered

**H&I Clinical Consultant Lead / Principal Clinical Scientist**
Sharon Vivers PhD DipRCPath

**Director of Quality and Regulation**
Salmah Ahmed

## 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@anthonynolan.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: **Complaints@anthonynolan.org**

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London NW3 2NU

Find out more at **anthonynolan.org**

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