This is an exciting time in genetics. New technologies are not only changing the way we test and diagnose genetic disease but are facilitating comprehensive analysis on a scale that was simply not feasible in the past. The same technologies applied in the research arena are forging new discoveries that shed light on the genetic basis of common and rare disorders, providing opportunities for new test development.

Our modern laboratory is well equipped with state of the art equipment that embraces new developments such as next generation sequencing, non-invasive prenatal testing and stratified medicine. With a team of highly-qualified and experienced staff, along with our strong focus on research, we have gained an international reputation for the delivery of a quality, accredited service and translation of research into improved patient care.

Professor Nick Cross
Use the colour coded pages together with the table on page 6 to guide you through the range of tests and services available.
Our service at a glance

- Established in 1967, the Wessex Regional Genetics Laboratory provides genetic diagnostic testing to a population of 2.7 million in south central England.

- In addition to patients from our local region, we welcome referrals from non-NHS and overseas patients. Please contact the laboratory administrator for further details.

- We offer an extensive range of tests including array-CGH, mutation detection, Next Generation Sequencing, karyotyping and specialised Prenatal and Oncology services. We issue over 10,000 diagnostic reports annually.

- Our modern well-equipped laboratory is based at Salisbury District Hospital. The laboratory is nationally registered (CPA accredited) and we participate in all relevant National and European EQA schemes.

- Our ongoing programme of research and development allows us to continually improve the range of tests available and to adapt to technological advances and service requirements.

Referrals by Category

- Wessex
- Other NHS
- UK Private
- Overseas

“We have been really pleased with the report emailing service and the ability to attach the PDFs to our fetal medicine database”

(Sally Boxall, Service User)
What you can expect from our service

- We welcome samples by post or courier service.
- Samples are unpacked and booked in by a dedicated technical team. Where appropriate, duty scientists carry out sample triage to ensure that the most efficient and effective investigations are initiated for each patient.
- Samples are stored in bar-coded tubes for safe and efficient sample tracking.
- All results and reports are checked and authorised by senior scientists.
- We provide detailed and fully interpreted reports, which can be e-mailed securely via ‘NHS.net’ accounts for direct uploading into patient notes.
- We strive to meet nationally recommended turn-around times in all areas of the laboratory.
- A duty scientist is always available for clinical liaison and to provide further information if required.
# Genetic and Genomic Diagnostic Services Overview

<table>
<thead>
<tr>
<th>Referral Category</th>
<th>Technique</th>
<th>Example conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perinatal testing</td>
<td>Rapid QF-PCR Karyotype Array-CGH</td>
<td>Prenatal diagnosis Fetal sexing Pregnancy loss</td>
</tr>
<tr>
<td>Reproductive genetic testing</td>
<td>Karyotype Cystic fibrosis screen Y microdeletions</td>
<td>Infertility</td>
</tr>
<tr>
<td>Genomic &amp; Molecular services / Congenital genetic conditions / Neonatal, paediatric and adult referrals (including late onset disorders and hereditary cancers)</td>
<td>Array-CGH Rapid QF-PCR (neonates)</td>
<td>Developmental delay / Dysmorphism</td>
</tr>
<tr>
<td></td>
<td>NGS: Gene panels, high-throughput screening</td>
<td>Breast cancer Marfan and aortopathies Lynch syndrome, Noonan spectrum, NF1</td>
</tr>
<tr>
<td></td>
<td>Targeted molecular testing, predictive and carrier testing</td>
<td>Common or specific mutations e.g. cystic fibrosis, neuromuscular disorders</td>
</tr>
<tr>
<td></td>
<td>Specialised molecular testing, MS-PCR, MLPA X-inactivation RNA splicing</td>
<td>Rare conditions Imprinting disorders e.g. Prader-Willi / Angelman</td>
</tr>
<tr>
<td>Oncology services</td>
<td>Karyotype, RT-PCR panel; targeted FISH + molecular tests</td>
<td>Haematological malignancies Solid tumours Hereditary cancers</td>
</tr>
</tbody>
</table>

For explanation of abbreviations used in the table, see glossary on page 22
Pre- & Perinatal Testing

- We provide a comprehensive prenatal diagnostic service that can be performed on either amniocentesis or chorionic villus samples. The testing approach is tiered depending upon the indication for testing.

- All referrals are provided with a QF-PCR analysis to detect targeted aneuploidies (e.g. trisomy 21 Down syndrome).

- Referrals presenting with abnormalities detected on ultrasound scan or a nuchal translucency >=3.5 mm are provided with a high-resolution analysis for genomic imbalances by array comparative genomic hybridisation (array-CGH).

- DNA can be isolated from prenatal samples to provide a diagnosis for a wide range of single gene disorders for referrals that have appropriate preparatory investigations.

- Conventional karyotyping and cell culture are also available when required.
Pregnancy loss
We provide a tiered testing strategy for the screening of genomic abnormalities responsible for pregnancy loss:
1. QF-PCR testing to detect the common aneuploidies.
2. DNA extraction and storage on all pregnancy losses allowing further genetic testing (single-gene disorders or array-CGH) as deemed appropriate by a multi-disciplinary team.

If requested, we can also provide cell culture for biochemical assays and/or the long-term storage of viable cells in liquid nitrogen from a wide range of tissue types.

Karyotypes are automatically captured and analysed with the aid of electronic imaging
Conventional karyotyping is available for patients referred with infertility and disorders of sexual development (DSD).

Molecular genetic screening for cystic fibrosis mutations and Y chromosome microdeletions is undertaken for azoo-spermic / oligospermic males.

Molecular genetic screening of specific genes involved in disorders of sexual development (including SRY, DAX1, CAH) is available.

Factor V Leiden testing is available for miscarriage couples.
Whole genome analysis is undertaken on neonatal, paediatric and adult referrals with developmental delay, autism spectrum disorder, moderate to severe learning difficulties, multiple congenital anomalies and dysmorphic features.

- **QF-PCR** is undertaken prior to whole-genome analysis where a diagnosis of a common trisomy or sex chromosome aneuploidy is suspected.
- **Array comparative genomic hybridisation (array-CGH)** is our first-line investigation for patients with developmental delay and dysmorphism or multiple congenital anomalies.
- **Conventional karyotyping** is undertaken on patients with non-specific clinical phenotypes without developmental delay.

**Array-CGH**

The WRGL was one of the first NHS labs to offer a full diagnostic array-CGH service and now provides a comprehensive service to the Wessex region, the Channel Isles and to private laboratories both within and outside the UK.

- We have gained extensive experience and expert knowledge in the interpretation and reporting of array-CGH-detected imbalances and have consequently developed a substantial data base which supplements both national and international CNV database resources.
- We undertake in-depth investigation of all imbalances detected, **only reporting anomalies likely to be of clinical significance** thus minimising patient anxiety.
We issue comprehensive reports listing the genes involved in the imbalances, the likely clinical significance and recurrence risks with references to relevant papers, literature and patient support groups. The quality of our reports has attracted many referrals from overseas.

Having access to probes from the Sanger Centre’s 30K clone set enables us to confirm and follow up array-CGH-detected imbalances with fluorescence in situ hybridisation (FISH). We can, therefore, detect or exclude a parental balanced chromosome rearrangement thus providing a more accurate recurrence risk. This service can be extended to other centres enabling the follow up of array-CGH imbalances detected elsewhere.

The WRGL is a founder member of the International Standards for Cytogenomic Array (ISCA) consortium. The ISCA array utilises 60,000 oligonucleotide probes in multiples of eight arrays per slide (8x60K format).
Fluorescence *in situ* hybridisation (FISH)

A panel of FISH probes is available for the detection of sub-microscopic chromosomal imbalances associated with the common microduplication/deletion syndromes, as well as for the elucidation of complex chromosome imbalances and confirmation of array-CGH detected imbalances. Please refer to our website for a complete list of probes available.

We use a variety of molecular genetic techniques to provide an extensive range of diagnostic tests, from Next Generation Sequencing of large gene panels to analysis of specific mutations by methylation or RNA splicing analysis.
Next-generation sequencing (NGS) involves large-scale parallel sequencing of millions of short DNA fragments. The technology provides the capacity to test multiple patients for many genes in a single run with sufficient depth of coverage to detect potentially causative sequence changes with high sensitivity.

We offer a range of **Next Generation Sequencing gene panels** including:

- Familial aortopathy and connective tissue disorders, including Marfan syndrome
- Cancer predisposition, including hereditary breast/ovarian cancer and Lynch syndrome
- RASopathies: developmental disorders of *RAS/MAPK* dysregulation, including Noonan syndrome spectrum and neurofibromatosis type 1

Further development of NGS panels is being implemented; please refer to our website for details of our current panels.
We offer screening for unknown mutations in large single genes, or multiple genes, for over 50 genetic conditions (see table below for examples). Our high-throughput screening facility utilises a combination of Next Generation Sequencing, bi-directional sequencing and MLPA dosage analysis to provide rapid, sensitive and cost-effective full gene screening.

In addition to clearly causative mutations, full screens frequently identify sequence variants of unknown clinical significance (VUS). We have considerable experience in the interpretation and reporting of these variants.

<table>
<thead>
<tr>
<th>Syndrome / Phenotype</th>
<th>Genes Tested</th>
</tr>
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<tbody>
<tr>
<td>Breast and Ovarian Cancer</td>
<td>BRCA1, BRCA2, RAD51C, RAD51D</td>
</tr>
<tr>
<td>Marfan Syndrome and TAAD</td>
<td>FBN1, TGFBR1, TGFBR2, ACTA, MYH11, SMAD3</td>
</tr>
<tr>
<td>Neurofibromatosis type I</td>
<td>NF1, SPRED1</td>
</tr>
<tr>
<td>Cowden syndrome, PTEN-related cancers</td>
<td>PTEN</td>
</tr>
<tr>
<td>Colon cancer (Lynch syndrome)</td>
<td>MLH1, MSH2, hMSH6</td>
</tr>
<tr>
<td>Noonan syndrome</td>
<td>PTPN11, RAF1, SOS1, SHOC2, KRAS</td>
</tr>
</tbody>
</table>
We offer targeted screening for many single-gene disorders where testing of a small number of common mutations can result in a high pick-up rate while avoiding the increased cost and turnaround time of a full gene screen. For example, the cystic fibrosis kit we use screens for mutations which account for over 90% of all CF mutations in populations of NW European origin and 71% of CF mutations worldwide.

Targeted screening can also be useful as a pre-screen for founder mutations, e.g. the Ashkenazi Jewish BRCA mutations. We can also confirm specific mutations identified in research laboratories or in unaccredited centres.

Following identification of a mutation in the index case, targeted testing allows the detection/exclusion of the mutation in any at-risk family members for both predictive testing (dominant conditions) and carrier testing (recessive conditions). Predictive testing for late onset conditions, such as Huntington disease, can be referred via a clinical geneticist.

Where appropriate, targeted testing can also include prenatal testing for familial mutations using DNA from amniotic fluid or chorionic villus samples.

DNA sequence trace showing single nucleotide substitution.
Specialised tests involve rare conditions or the identification of classes of mutation not detectable by standard techniques, e.g. imprinting disorders, uniparental disomy, repeat expansions and MLPA dosage analysis. Alternatively, some specialist tests can be used to follow up mutations to assess their clinical significance, e.g. RNA analysis, X-inactivation studies.

**MLPA dosage analysis**
Multiplex Ligation-Dependent Probe Amplification (MLPA) can be used as part of a full gene screen, or alternatively as a targeted test for microdeletion syndromes (e.g. Di George syndrome) or for smaller imbalances within a gene (e.g. deletions or duplications in the dystrophin gene which cause DMD/BMD). For further information, see [www.mlpa.com](http://www.mlpa.com)

![Deletion identified by MLPA](image)

**RNA analysis**
For genes expressed in blood cells (e.g. *BRCA1*, *BRCA2* and *FBN1*), it is possible to assess whether specific sequence changes affect RNA splicing. Abnormal transcripts can be detected using primers in exons flanking the sequence change; sequencing the abnormal transcript then confirms that it is associated with the sequence change.
Triplet repeat analysis: PCR, TP-PCR and Southern blotting
Many common genetic disorders are caused by expansions in repeat sequences either within or closely adjacent to genes, such as the trinucleotide repeat CGG in the FMR1 gene causing Fragile X Syndrome. These are tested for by fluorescent PCR analysis supplemented by triplet-primed PCR (TP-PCR) and Southern blotting for very large expansions.

X-inactivation testing
X-inactivation patterns in females can be determined at two loci on the X chromosome. This can be used as a surrogate test for an X-linked inheritance pattern or to assess the clinical significance of X-linked CNVs frequently identified by array-CGH.

Imprinting disorders
Numerous genetic conditions are associated with imprinting defects and we offer testing for the following syndromes:

- Prader-Willi/Angelman syndromes
- Russell-Silver syndrome
- Beckwith-Wiedemann syndrome
- Temple/Kagami-Ogata syndromes (Chromosome 14q)
- Transient Neonatal Diabetes Mellitus (TNDM)
- Pseudohypoparathyroidism

TNDM is tested free of charge as part of a University of Southampton research project (IDFOW: Imprinting Disorders, Finding Out Why) which is based within the WRGL. Visit the website for more information: www.southampton.ac.uk/geneticimprinting/informationpatients/imprintingfindingoutwhy.page

We also hold a large bank of fluorescent microsatellite primers enabling us to offer testing for uniparental disomy (UPD) of any chromosome.
The WRGL offers a comprehensive service to aid the diagnosis, monitoring and management of a wide range of haematological malignancies, solid tumours and an expanding number of hereditary and somatic mutation cancers. Our results are incorporated into the integrated MDT report for patient management.

**Haematological malignancies**

Our integrated service for AML, ALL, CML and other MPN (including \textit{JAK2}, \textit{CALR} and \textit{MPL} testing), MDS, and lymphoproliferative disorders incorporates karyotyping, FISH and molecular tests as appropriate. We offer \textit{targeted FISH testing} for a range of haematological malignancies including multiple myeloma, CLL, and NHL including BL and DLBCL. This may be performed on cultured cells, smears, lymph node dabs, selected plasma cells, or paraffin-embedded tissue sections (PETs) as appropriate.

Targeted molecular tests include an \textit{RT-PCR panel} to detect frequent gene rearrangements associated with ALL and AML and \textit{BCR/ABL1} kinase mutations.

\begin{center}
\textbf{Oncology}
\end{center}

\begin{tabular}{|c|c|c|c|}
\hline
1 & 2 & 3 & 4 \\
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5 & 6 & 7 & 8 \\
\hline
9 & 10 & 11 & 12 \\
\hline
13 & 14 & 15 & 16 \\
\hline
17 & 18 & 19 & 20 \\
\hline
21 & 22 & X & Y \\
\hline
\end{tabular}

\textit{t(4;22), BCR-PDGFR\textsubscript{A} rearrangement c/w atypical CML}
Oncology Research and Development

The WRGL has an international reputation for the study of myeloproliferative disorders and myeloma thanks to research programmes carried out under the leadership of Professor Nick Cross and Dr Fiona Ross respectively.

Professor Nick Cross’s Leukaemia and Lymphoma Research group’s primary interest is in the use of state-of-the-art technology to identify novel causative gene mutations thereby leading to a greater understanding of basic biology and the development of novel therapies. Translation of this research has led to routine molecular diagnostics for MPNs such as JAK2, BCR/ABL1 kinase, cKIT and PDGFRA mutation testing.

As part of the Myeloma IX trial Dr Fiona Ross and her team worked out the relationship of FISH results to the international scoring system to establish a prognostic index. We are now translating this research into routine service, offering the most comprehensive myeloma FISH service in the country.

<table>
<thead>
<tr>
<th>Myeloma FISH probes</th>
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<tbody>
<tr>
<td>IGH/FGFR3</td>
<td>t(4;14)</td>
</tr>
<tr>
<td>IGH/CCND1</td>
<td>t(11;14)</td>
</tr>
<tr>
<td>IGH/MAF</td>
<td>t(14;16)</td>
</tr>
<tr>
<td>TP53/17cen</td>
<td>17p</td>
</tr>
<tr>
<td>CDKN2C/CKS1B</td>
<td>1p/1q</td>
</tr>
</tbody>
</table>

i) Purified plasma cells; ii) FGFR3/IGH fusion; iii) CKS1B gain
Solid Tumours
We provide a targeted FISH service for solid tumours which may be performed on PETs or fresh biopsy touch preparations. This is recommended for a number of tumours, e.g. neuroblastoma, Ewings sarcoma, rhabdosarcoma, synovial sarcoma and other soft tissue sarcomas where genetic abnormalities have been related to diagnosis or prognosis. We also offer a solid tumour karyotyping service for paediatric tumours.

We offer targeted somatic mutation testing of an increasing number of genes involved in tumorigenesis for diagnosis, prognosis and therapy options (detailed in Stratified Medicine).

Hereditary Cancers
Full-screen and predictive testing is available for an increasing list of cancer-predisposing genes; for a full list, visit our website.
We have developed a strategy using Next Generation Sequencing to detect specific mutations that are common in the population.

This can be used to detect mutations in common genetic disorders, such as the p.(Cys282Tyr) mutation in hereditary haemochromatosis, or the S and Z alleles in alpha-1-antitrypsin, and additionally it has the sensitivity to detect mutations commonly acquired in haematological proliferative neoplasia such as the JAK2 p.(Val617Phe) and JAK2 exon 12 mutations, CALR insertion/deletion mutations and MPL mutations in codons 505 and 515.

This strategy can be employed to develop further tests as further associations and discoveries are made.
<table>
<thead>
<tr>
<th>Glossary</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Array-CGH</td>
<td>Array comparative genome hybridisation</td>
</tr>
<tr>
<td>CAH</td>
<td>Congential adrenal hyperplasia</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>CNV</td>
<td>Copy number variant</td>
</tr>
<tr>
<td>CPA</td>
<td>Clinical Pathology Accreditation</td>
</tr>
<tr>
<td>DMD/BMD</td>
<td>Duchenne &amp; Becker muscular dystrophy</td>
</tr>
<tr>
<td>DSD</td>
<td>Disorders of Sexual Development</td>
</tr>
<tr>
<td>EWS</td>
<td>Ewing’s sarcoma</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence <em>in situ</em> hybridisation</td>
</tr>
<tr>
<td>ISS</td>
<td>International scoring system</td>
</tr>
<tr>
<td>MDT (report)</td>
<td>Multi-disciplinary team</td>
</tr>
<tr>
<td>MLPA</td>
<td>Multiplex Ligation-Dependent Probe Amplification</td>
</tr>
<tr>
<td>MPN</td>
<td>Myelo proliferative neoplasm</td>
</tr>
<tr>
<td>MS-PCR</td>
<td>Methylation sensitive – Polymerase Chain Reaction</td>
</tr>
<tr>
<td>NGS</td>
<td>Next Generation Sequencing</td>
</tr>
<tr>
<td>NIHR</td>
<td>National Institute for Health Research</td>
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<tr>
<td>NIPT</td>
<td>Non-invasive prenatal testing</td>
</tr>
<tr>
<td>PETS</td>
<td>Paraffin-embedded tissues</td>
</tr>
<tr>
<td>QF-PCR</td>
<td>Quantitative Fluorescence - Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RAPID</td>
<td>Reliable Accurate Prenatal non-Invasive Diagnosis</td>
</tr>
<tr>
<td>RNA splicing</td>
<td>Ribonucleic acid splicing</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription – Polymerase Chain Reaction</td>
</tr>
<tr>
<td>TNDM</td>
<td>Transient Neonatal Diabetes Mellitus</td>
</tr>
<tr>
<td>TP-PCR</td>
<td>Triplet primed PCR</td>
</tr>
<tr>
<td>UPD</td>
<td>Uniparental disomy</td>
</tr>
<tr>
<td>VUS</td>
<td>Variant of unknown significance</td>
</tr>
</tbody>
</table>
Quality and Excellence

We are a fully-accredited laboratory genetics service and are committed to providing users with a service of the highest quality. We operate to stringent technical and administrative standards and are accredited by CPA. We are awaiting a transition assessment to ISO15189. We are committed to providing users with a service of the highest quality.

We operate a comprehensive quality management system and aim to produce:

- Accurate results
- Using appropriate testing strategies
- In an appropriate timeframe
- With appropriate comment and interpretation to assist clinicians in providing the best management of their patients.

As part of our ongoing commitment to quality standards we participate in all relevant UKNEQAS (UK National External quality assessment scheme) and EMQN (European Molecular genetics Quality Network) schemes.

Accredited since 1988 - lab No. 1175
Contacting the Laboratory

Our website has full details of sample requirements, storage and packing information as well as referral forms for download. You can also contact the laboratory during office hours: 9.00 am to 5.30 pm Monday to Friday. Please leave a message on the answer machine out of hours, or e-mail (see below).

Address
Wessex Regional Genetics Laboratory
Salisbury NHS Foundation Trust
Salisbury District Hospital
Odstock Road
Salisbury
Wiltshire
SP2 8BJ
United Kingdom

Phone +44 (0)1722 429080
Fax +44 (0)1722 331531

Email (general enquiries to duty scientist)
shc-tr.WRGLdutyscientist@nhs.net

Website
www.wrgl.org.uk