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A partnership between Newcastle University and Newcastle Hospitals NHS Foundation Trust

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Clinical Services
Information for Users

October 2017

Details of all services are also available at www.newgene.org.uk or email: *info@newgene.org.uk*

NewGene Ltd uses high throughput genotyping, DNA sequencing and real time PCR to provide clinical laboratory services, principally to haematology, pathology and clinical genetics departments.

Laboratory opening hours:

The laboratory is staffed between 8.30am and 5.00pm Monday to Friday, excluding Bank Holidays.

NewGene is accredited with the United Kingdom Accreditation Service (UKAS) to ISO standard 15189 (medical laboratories). Tests marked * below, have recently been introduced into the test repertoire and lie outside the current scope of accreditation.

Clinical scientists are registered with the Health and Care Professions Council.

The laboratory participates in the following external quality assurance schemes:

- UK NEQAS for Molecular Genetics
- European Molecular Quality Network (EMQN)
- UK NEQAS for Leucocyte Immunophenotyping

For tests where no formal EQA scheme exists, an informal method of proficiency testing is in place.

List of tests and sample requirements

Alport syndrome* - full gene sequencing using next generation sequencing technology

Whole blood samples in 4.5 ml EDTA tubes or 4 ug of DNA (260/280: 1.8-2.0).
Genes included in the assay are *COL4A3, COL4A4, COL4A5.*

Aortopathy* (Marfan and related syndromes) gene panel – full gene sequencing using next generation sequencing technology

Whole blood samples in 4.5 ml EDTA tubes or 4 ug of DNA (260/280: 1.8-2.0).
Genes included in the assay are *FBN1, TGFBR1, TGFBR2, ACTA2, SMAD3, MYH11, COL3A1, EFEMP2 (FBLN4), FBN2, MYLK, NOTCH1, SLC2A10, FLNA, TGFB2, SKI.*

BCR-ABL fusion gene targeted mutation analysis

Whole blood samples in 4.5 ml EDTA tubes, which must arrive at the NewGene lab within 48 hours of the sample being taken.

BCR-ABL fusion gene monitoring in chronic myeloid leukaemia

This test requires RNA and therefore whole blood samples should be collected and arrive at the NewGene laboratory within 48 hours of the sample being taken.

BRAF targeted mutation detection (colorectal cancer, melanoma, hairy cell leukaemia)

Whole blood samples in 4.5 ml EDTA tubes for hairy cell leukaemia.
Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks, cut curls (minimum of 5 x 10 µm thick curls) or cytology slides. Ideally tumour content should be 30% of the specimen.

BRCA1 and *BRCA2* full gene sequencing in hereditary breast cancer – full gene sequencing using next generation sequencing technology

Whole blood samples in 4.5 ml EDTA tubes *or* extracted DNA (2 µg total quantity at >50 ng/µl). Copy number analysis using MLPA is performed by the Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust.

BRCA1 and *BRCA2* full gene sequencing in somatic cancer samples* – full gene sequencing using next generation sequencing technology

Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks, cut curls (minimum of 5 x 10 µm thick curls) or cytology slides. Ideally tumour content should be 30% of the specimen.

cKIT targeted mutation analysis (D816V mutation in mastocytosis and acute myeloid leukaemia, exons 8 and 17)

Whole blood samples or bone marrow in 4.5 ml EDTA tubes. Mast cell enrichment is recommended for samples referred for mastocytosis testing.

Note – see below for *cKIT* mutation analysis in GIST.

cKIT and *PDGFRA* mutation analysis in gastrointestinal stromal tumours (GIST)

Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks, cut curls (minimum of 5 x 10 µm thick curls) or cytology slides. Ideally tumour content should be 30% of the specimen.

EGFR and *KRAS* targeted mutation detection (non-small-cell lung cancer)

Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks, cut curls (minimum of 5 x 10 µm thick curls), cytology slides or fixed cell suspensions. Ideally tumour should be histologically classified as adenocarcinoma and tumour content should be 30% of the specimen.

Familial hypercholesterolemia

Whole blood samples in 4.5 ml EDTA tubes. Targeted mutation detection of 20 selected mutations in *LDLR*, *APOB* and *PCSK9*. Also, detection of 12 SNPs in various key genes that allow calculation of a 'SNP-score' which further refines the likelihood of polygenic or monogenic disease.

Samples that are negative in the mutation detection test will be fully sequenced using next generation sequencing technology, for the genes *LDLR*, *AOE*, *PCSK9* and selected regions of *APOB*, where clinically indicated by the regional lipid clinic MDT.

Familial adenomatous polyposis (FAP)*

Whole blood samples in 4.5 ml EDTA tubes or 4 ug of DNA (260/280: 1.8-2.0). Full gene sequencing using next generation sequencing technology of the coding regions of the *APC* and *MUTYH* genes. Copy number analysis using MLPA is referred to the Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust.

Hereditary non-polyposis colorectal cancer*

Whole blood samples in 4.5 ml EDTA tubes or 4 ug of DNA (260/280: 1.8-2.0). Full gene sequencing using next generation sequencing technology, of the coding regions of four genes: *MLH1*, *MSH2*, *MSH6* (excluding exon 1) and *PMS2*, and sequencing of the 3' untranslated region of *EPCAM*. Copy number analysis using MLPA is referred to the Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust.

JAK2 V617F *mutation, JAK2* exon 12 mutations and *MPL* and Calreticulin (*CALR*) targeted mutation detection in myeloproliferative neoplasms

Whole blood samples in 4.5 ml EDTA tubes

KRAS and *NRAS* targeted mutation detection in colorectal tumours

Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks, cut curls (minimum of 5 x 10 µm thick curls) or cytology slides. Ideally tumour content should be 30% of the specimen.

Marfan syndrome*

Whole blood samples in 4.5 ml EDTA tubes or 4ug of DNA (260/280: 1.8-2.0). Full gene sequencing using next generation sequencing technology, of the coding region of the *FBN1* gene.

Medulloblastoma subgroup classification* (results issued on a research basis only)

This test examines the methylation signature of DNA extracted from medulloblastoma and determines a subgroup classification (MB_{WNT}, MB_{SHH}, MB_{Grp3} or MB_{Grp4}) using the web app, MIMIC* v3.3.4 (MInimal MethylatIon Classifier). MIMIC has not been validated for clinical use and is for research use only.

Formalin fixed paraffin embedded (FFPE) samples in the form of tumour blocks or cut curls (minimum of 5 x 10 µm thick curls), or fresh tissue samples. Ideally tumour content should be >50% of the specimen.

Samples must have a confirmed histopathological diagnosis of medulloblastoma.

Noonan spectrum of disorders (RASopathies)

Sanger sequencing of the PTPN11 gene - exons 3, 4 and 8 as a pre-screen, followed by full gene sequencing using next generation sequencing technology for the following genes - *PTPN11, SPRED1, KRAS, SOS1, RAF1, NRAS, BRAF, SHOC2* (exon 1 only)*, MAP2K1, MAP2K2, HRAS, CBL, NF1, RIT1* and *A2ML1*
Whole blood samples in 4.5 ml EDTA tubes *or* extracted DNA (5µg total quantity at >50ng/µl,; 260/280: 1.8-2.0).

TPMT genotyping

Whole blood samples in 4.5 ml EDTA tubes

TP53 full gene sequencing

Whole blood samples in 4.5 ml EDTA tubes *or* extracted DNA (1 µg total quantity at >50ng/µl; 260/280: 1.8-2.0).

Referral procedure

1. All sample tubes must be labelled with the following minimum information:

   Full name of the patient
   Date of birth of the patient

2. Pathology blocks must be labelled with the histopathology block reference of the referring laboratory.

3. All samples should be accompanied by a test request form. These are available as downloads from the NewGene web site or on request (0191 242 1923).

   If it is not possible to use a referral form, the required information should be provided in a letter.

   The information provided must include:

   Full name of the patient
   Date of birth
   Sex of the patient
   NHS number
   Patient's postcode
   Tests required
   Name of referring clinician, address for issue of report and contact telephone number

   Samples which do not conform to these criteria may be discarded.

   Ensure that the name and date of birth on the card are exactly the same as those on the sample tube.

   Special care should be taken to ensure that forenames and surnames are clearly distinguished.

Sample transport

Specific referral instructions for the different tests are given on the relevant referral forms.
Samples should be delivered as follows:

By courier, taxi or hospital delivery van as soon as possible after the sample is taken to

NewGene Ltd
Sample Reception laboratory
Institute of Human Genetics
Central Parkway
Newcastle upon Tyne
NE1 3BZ

Or, by post to

NewGene Ltd
Bioscience Building
International Centre for Life
Newcastle upon Tyne
NE1 4EP

Note: Referrals for myeloproliferative neoplasm tests (JAK2, MPN and calreticulin) should be sent to the Specials Laboratory, Haematology Dept., Leazes Wing, Royal Victoria Infirmary, Newcastle upon Tyne.

Sample packaging

Samples should be properly packaged by the referrer. Packaging should be strong and of good quality. In addition to the sample receptacle, here should be two further layers of packaging that should be closed and sealed to prevent any loss of contents. There should be sufficient cushioning to prevent breakage of the primary receptacle. The outer layer of packaging should be rigid. Please contact the laboratory (0191 242 1923) with any queries, and see:

http://www.hse.gov.uk/aboutus/meetings/committees/acdp/050208/acdp88p6.pdf .

Note: Special delivery bags are available on request for KRAS referrals only

High risk samples should be clearly labelled in an appropriate manner (see below).

Hazardous samples

Samples known or suspected to be microbiological hazards **must** be identified as such by the use of appropriate hazard warning labels. Please contact the laboratory before referring samples from patients where a prion-related disorder is suspected.

Consent for testing and data storage

Consent for DNA testing must be obtained from the patient by the referring clinician prior to referral of the sample. This is not the responsibility of the NewGene laboratory staff.

Personal data is stored by the laboratory but is only used for analytical and reporting purposes associated with the required test.

Referral laboratories

The vast majority of the tests in the NewGene repertoire are performed in the NewGene laboratory. The exceptions are:

(1) Copy number analysis of BRCA1 and BRCA2 which forms an important part of the BRCA full gene sequencing test, is performed by the Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust, using MLPA analysis, when required.

(2) Samples that require Sanger sequencing are PCR amplified at NewGene, but then transferred to the Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust, for the Sequencing reactions to be carried out. After capillary analysis, raw data is returned to NewGene.

The sequencing data is analysed by NewGene staff prior to reporting.

The Northern Genetics Service is a CPA accredited laboratory (reference 2212). It is not currently accredited to ISO 15189 and therefore, the subcontracted work referred to above is NOT covered by UKAS accreditation.

Contacts

Laboratory address:

NewGene Ltd
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NE1 4EP
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Scientific Business Development Manager:

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Advice concerning samples, test results and assay performance

Advice is available from registered Clinical Scientists, Dr Ann Curtis (ann.curtis@nuth.nhs.uk), and Dr Louise Johnston (louise.johnston@nuth.nhs.uk), tel 0191 242 1923

Where appropriate, information about the sensitivity, specificity and performance of assays is provided in the clinical report along with any factors that may influence the interpretation of results. The following general points should also be noted:

Timeliness of receipt of samples – samples for *BCR-ABL* monitoring or *BCR-ABL* fusion gene mutation analysis must arrive at the laboratory within 48 hours of the sample being taken in order that high quality RNA can be obtained. Other samples, where the analyte is DNA, are stable at 4°C for up to 5 days.

Tumour content - FFPE samples should ideally contain at least 30% tumour material. It is important that the estimated tumour content is supplied with the referral form.

Test sensitivity - For all molecular pathology and molecular haemato-oncology tests, the sensitivity of mutation detection is 5% on a wild type background unless otherwise noted.

Next generation sequencing tests – A minimal coverage of 50-fold is guaranteed for key genes in all gene panel tests. Regions of reduced coverage for key genes will be filled in using Sanger sequencing. Specific details are available on request.

Tests that generate a quantitative result are reviewed with respect to the uncertainty of measurement associated with the numeric value. Specific test information is available on request.

Comments and complaints

We aim to deliver consistently high standards of care. We are always pleased to hear of anything you have to say and any suggestions you might have to help us continuously improve our services. If you have complaints about our service these will be dealt with promptly according to our customer complaints procedure. Please contact us by telephone, e-mail or letter. Contact details are at the beginning of this information sheet.

Further information*:*

Visit the web site: www.newgene.org.uk or email: ***info@newgene.org.uk***
Telephone: +44 (0)191 242 1923