Guidance document:

Rapid Exome Sequencing for acutely unwell children with a likely monogenic disorder

NHS England and NHS Improvement

Rapid Exome Sequencing for acutely unwell children with a likely monogenic disorder

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Document management

Revision history

Version	Date	Author	Summary of changes
4.0	12/06/2020	As above	Updates in response to COVID-19

Reviewers

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Reviewer name	Title/responsibility	Date	Version

Approved by

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Contents

Introduction

The Genomic Medicine Service (GMS) provides the national genomic testing for rare disease and cancer within NHS England by delivery of the National Genomic Test Directory.

This Clinical Policy defines expectations of the rapid exome sequencing service which will be provided by the network of Genomic Laboratory Hubs (GLHs) as part of the Genomic Medicine Service (GMS). While all tests may need to be offered with a rapid turnaround time under specific circumstances, this particularly applies to clinical indication R14, acutely unwell children with a likely monogenic disorder, within the National Genomic Test Directory where utility is always tied to rapid results.

The R14 clinical indication (acutely unwell children with a likely monogenic disorder) will be tested by whole genome sequencing (WGS) when a clinically relevant turnaround time (as defined by NHS England) is achievable. In the interim period a rapid exome sequencing service will be provided by an NHS England designated laboratory within the GMS.

Background

Testing for acutely unwell children (neonatal intensive care unit patients [NICU] and paediatric intensive care unit patients [PICU]) with a likely monogenic disorder is currently available as an NHS rapid trio exome service at the Royal Devon & Exeter NHS Foundation Trust, Exeter (South West GLH) and is used by Clinical Genetics services throughout England. Research studies in Cambridge and London (Great Ormond Street Hospital) have used whole genome sequencing (WGS) to provide rapid genetic diagnosis for clinical management of NICU/PICU patients (French *et al.,* 2019; Mestek-Boukhibar *et al.,* 2018). All three bioinformatics pipelines include analysis of single nucleotide variants (SNVs), small insertions and deletions (<50 base pairs in size) and copy number variants (CNVs).

Whole exome sequencing (WES) has been identified as the most appropriate interim approach for rapid genetic diagnosis of NICU and PICU patients. Whole exome sequencing has a high diagnostic yield (36.9% for the first 1,232 cases tested in Exeter) and is more future-proof than a clinical exome sequencing approach that is restricted to a subset of known disease genes.

Service Provision

In summary:

- Whole exome sequencing (WES) will be performed for diagnostic purposes.
- Cases reviewed by the Testing GLH prior to the Home GLH sending samples, with input from the Home GLH designated Clinical Genetics Lead where appropriate.
- Cases where testing is likely to provide an immediate change in clinical management for the patient. In exceptional circumstances if clinically indicated, testing may be performed where results may affect family management.
- Where imminent demise of the patient is predicted then rapid exome sequencing will not be performed. As above in exceptional circumstances, if clinically indicated, testing may be performed where results may affect family

management.

- Standard diagnostic testing will be carried out in parallel (e.g. microarray) where indicated.
- Exome sequencing is not suitable for the detection of triplet repeat expansions, imprinting disorders and the presence of pseudogenes may prevent the detection of pathogenic variants. For disorders such as Spinal Muscular Atrophy, Congenital Myotonic Dystrophy, Prader-Willi, Angelman or Congenital Central Hypoventilation Syndrome please request testing from the home GLH using alternative technologies. Mitochondrial DNA rearrangements and low levels of heteroplasmy are not detectable by exome sequencing and testing should be requested from a specialist laboratory if a mitochondrial disorder is suspected.
- Trio testing where possible, with duos or singletons accepted according to sample availability.
- If there is insufficient DNA available from the proband for exome sequencing, it is possible to test for an autosomal recessive aetiology using parental samples (Stals *et al.,* 2018).
- All relevant familial samples, HPO terms and pedigree to be supplied at point of referral of the proband.

Eligibility Criteria

Clinical indication	**Testing Criteria**	**Stage in pathway for testing**	**Test requesting specialties**
R14	Acutely unwell children with a likely monogenic disorder.	Following discussion with Clinical Genetics, the child's local management team and the Testing laboratory.	Clinical Genetics

Table 1 - Testing criteria for this clinical indication are as listed in version 1 of the National Genomic Test Directory.

- Provision is also needed for families where a diagnosis is needed for management of a current pregnancy. This clinical indication will fall under R14. For example, testing for a pregnant patient in whom a genetic diagnosis is likely to inform her clinical management during the pregnancy or delivery. Alternatively, a couple may seek testing in order to find a diagnosis for their child, or a previously affected pregnancy, that enables prenatal testing for their current pregnancy.

Testing Pathways

Summary

During the interim period whilst WGS for NICU and PICU pathways is being implemented to meet the clinically relevant turnaround time, no new services will be employed. Based on current service provision and previous research and translational experience, the Royal Devon & Exeter NHS Foundation Trust, Exeter (South West GLH) has been assigned this service.

Role of the NICU/PICU/ICU team

The NICU/PICU/ICU team or Clinical Geneticist will complete the R14 test request form including patient details, HPO terms and email addresses and contact telephone number for the designated Clinical Geneticist lead and NICU/PICU/ICU requesting clinician (see Appendix 1). The completed request form is then emailed using a nhs.net email account to the testing laboratory by the clinical team, copying to the clinical geneticist and Home GLH. If the proband is affected with COVID-19 then this should be indicated on the referral form.

Role of the Home GLH

The role of the Home GLH (where the proband has been referred, Figure 1) is as follows:

- Processing of all familial samples
- DNA extraction from blood samples. This is the preferred sample type
- If blood is not available then Oragene saliva samples can be sent directly to the Testing GLH for the duration of the COVID-19 pandemic, if requested by the Home GLH to facilitate obtaining a rapid result. Blood samples can also be sent to directly to the Testing GLH if requested by the Home GLH.
- QC concentration: A minimum of 1µg of DNA (NanoDrop quantitation) must be sent for testing. If this is not available, then the Home GLH must communicate with the testing GLH to determine if the DNA sample available is suitable for exome sequencing.
- Dispatch of samples to testing GLH by courier service or first-class post as soon as all familial samples are ready for dispatch
- Participation in Management Information data collection

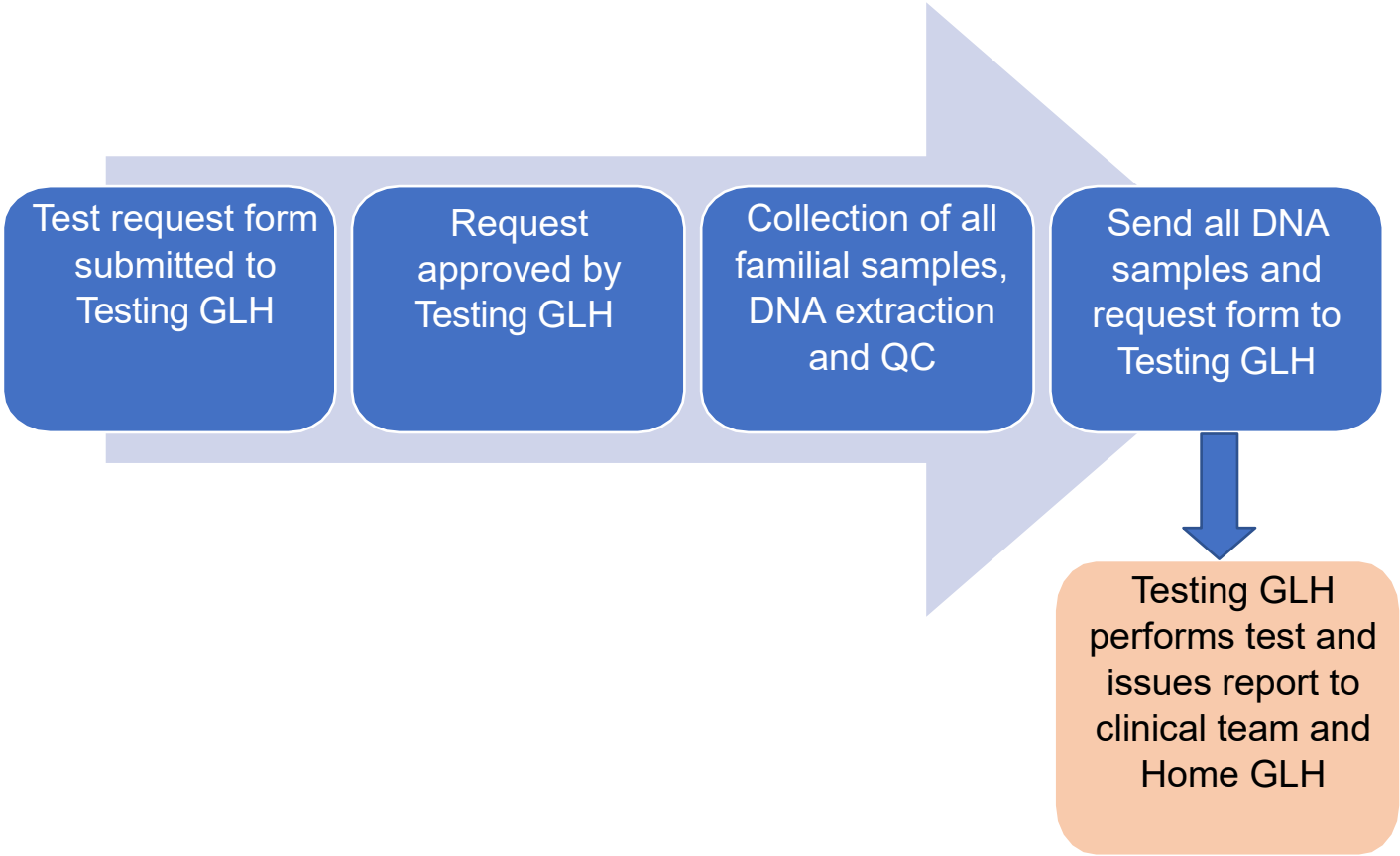


Figure 1 – Summary of the role of the Home GLH

Role of the Testing GLH

The role of the Testing GLH is as follows:

- Provision of an ISO 15189:2012 accredited service for rapid whole exome sequencing (WES) for referrals outlined in Section 3.
- Pre-log the request for testing upon receipt of the completed request form.
- Discussion with referring clinical team if required.
- E-mail confirmation of test suitability to NICU/PICU/ICU team and Home GLH designated Clinical Geneticist.
- Interpretation of the WES data and issue of fully clinically interpreted reports in collaboration with the Home GLH designated Clinical Geneticist and referring PICU/NICU/ICU team if required.
- Participation in Management Information data collection

Analysis

- Where a trio has been sequenced, analysis for referrals will primarily use a gene-agnostic family-based approach, although appropriate virtual PanelApp gene panels (https://panelapp.genomicsengland.co.uk/) will be applied where relevant. SNVs, small indels and CNVs must be analysed with the ability to detect compound heterozygous SNV or indel *in trans* with a CNV
- Gene-agnostic analysis initially *de novo*, compound heterozygous, homozygous and X-linked (males only) rare variants.
- Gene panel analysis based on HPO terms for singletons and duos
- Gene panel analysis where appropriate as a supplementary analysis for trios with no likely disease-causing variant to identify a heterozygous variant inherited from an unaffected parent
- Analysis will also include uniparental disomy and mitochondrial DNA variants

Reporting pathways

The Testing GLH will communicate the result to the referring Clinician and Clinical Geneticist by email. If no likely disease-causing variant is identified, a formal report to this effect will be issued to the requesting Clinician and Clinical Geneticist with a copy to the Home GLH.

If a variant (or variant pair) is identified that is considered likely causative, the Testing GLH will communicate details of the variant(s) with a request for feedback from the clinical team as to whether this is considered a plausible diagnosis and whether it explains the entire phenotype. Following variant confirmation by an orthogonal method (where appropriate), a formal report will be issued. The majority of variants will not require validation, but this will be necessary for some CNVs, variants within regions of low read depth or where there is uncertainty regarding the variant call(s).

In the situation where the identified variant(s) or variant pair(s) identified requires discussion with the referring Clinician and Clinical Geneticist, the email from the Testing GLH will include details of the variant(s), current evidence for variant classification, relevant publications and links to websites such as OMIM. This email initiates a multi-disciplinary discussion that can also involve external experts. Where required, a teleconference or WebEx will be arranged for the clinical team and Testing GLH (and any external experts) to discuss the case, decide upon any further testing or investigations, and agree the variant classification for the report.

The Testing GLH will comply with the ACGS practice guidelines for variant interpretation and reporting.

Incidental findings

Testing will focus on identifying disease-causing variants of direct relevance to the clinical referral and additional findings will not be actively sought. The testing strategy aims to reduce the likelihood of identifying pathogenic variants that predispose to other rare diseases, but the possibility of incidental findings cannot be excluded. Such findings may be discussed with the referring clinician on a case-by-case basis.

Turnaround times

Stage	Start of stage	End of stage	Target TAT (calendar days)
Testing GLH activity 1	All samples and required data received from Home GLH	Issue of preliminary result for patients for probands with possible clinically relevant finding or issue of final report for probands with no detected clinically relevant finding	14
Testing GLH activity 2	All samples and required data received from Home GLH	Issue of final report for probands with confirmed clinically relevant finding	21

Table 2 – Turnaround times

Roles and Responsibilities

Rapid sequencing services rely heavily on appropriate patient selection to ensure capacity is focused on those with the greatest likelihood of benefit. Clinical Geneticists will liaise with NICU/PICU/ICU teams to support implementation of the service, advise on the likelihood of a monogenic disorder and offer follow-up testing/counselling/management advice where appropriate. Clinical Scientists will be involved in case review, variant interpretation and reporting. Multi-disciplinary team working between the NICU/PICU/ICU teams, Clinical Geneticists and Clinical Scientists will be required for interpretation and reporting of complex cases. For COVID-19 severely affected individuals, multi-disciplinary team working may benefit from the inclusion of Immunologists and Infectious Disease Specialists.

R14 – rapid sequencing considerations

Consent

The following points should be discussed by the Clinical team with the parents prior to sequencing:

- It is possible that there may be no diagnosis after testing
- A result may be obtained that is difficult to interpret and will still leave some uncertainty
- Trio exome sequencing will reveal possible non-paternity (or non-maternity)

and this result would be discussed with the referring clinician.

- As parental samples are being sequenced as well as the child/baby findings may be identified that could affect the parents' own health. If this is the case, then these will be discussed with the parents
- This is a very new area and the understanding of DNA sequences is improving all the time. Result disclosure and post-test counselling will be based on knowledge that is current at the time of result interpretation. Potential changes over time are likely to occur in our knowledge of disease genes, pathogenicity of sequence variants and patient phenotypes. This means that reanalysis of the sequencing data at a later time may reveal the causative mutation.

Review

It is anticipated that over time, the testing criteria and requesting specialties for R14 referrals will be kept under review; these services will be evaluated regularly during implementation and rolled out more broadly where evidence indicates that this can be achieved effectively.

Move to Whole Genome Sequencing

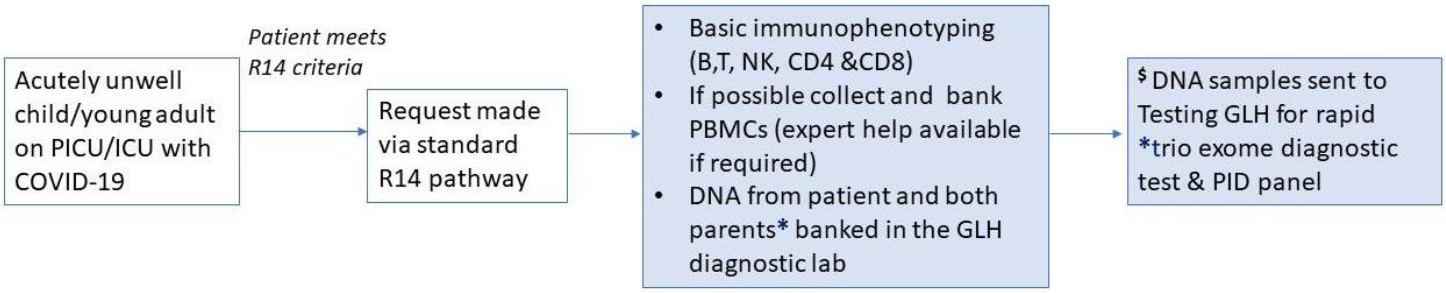
Data is available to demonstrate the benefit of whole genome sequencing for diagnosis in acutely unwell children (French *et al.,* 2019). Whole Genome Sequencing of R14 referrals will be implemented within 18 months of the rapid exome sequencing service going live within the GMS. The exact timings of this change will be dependent upon the implementation work with East Midlands and East of England GLH, South West GLH, NHS England and the GMS WGS provider to validate an appropriate clinical service meeting relevant turnaround times.

R14 testing for children with confirmed or suspected COVID-19

- Acutely unwell children and young adults (aged <21 years) with a clinical presentation thought potentially due to COVID-19 infection where an expedited diagnosis would help with their/family members immediate clinical management are eligible for the R14 test indication, unless there is an already established underlying genetic diagnosis or risk factors (e.g. cystic fibrosis or cardiorespiratory compromise due to congenital heart disease).
- Children with clinical features and blood markers entirely compatible with Paediatric Multisystem Inflammatory Syndrome temporally associated with COVID-19 (PIMS-TS), are unlikely to have a monogenic disorder, recruitment to the GenOMICC study would be more appropriate than R14 testing for these children (see below). There are a subset of immunodeficiency/immune dysregulation disorders that may be difficult to discriminate from PIMS-TS (e.g. primary HLH - Hemophagocytic Lymphohistioytosis). Clinical judgement will be required to determine those patients for whom the R14 test may be appropriate. Clinical indicators that may suggest a monogenic aetiology include syndromic features, personal or family history of severe/unusual infection, early onset severe autoimmunity and failure to thrive. Discussion with an expert in the appropriate clinical area may be helpful prior to referral (e.g. paediatric immunologist).
- Evidence of a positive COVID-19 RNA test is not an eligibility requirement.
- Gene panel testing (e.g. primary immunodeficiency disorder testing) does not need to be carried prior to referral, a bespoke diagnostic analysis strategy will

be undertaken for these children.

- Basic immunophenotyping of lymphocyte subsets (B, T and NK cells) and immunoglobulins should be carried out in parallel to referral for WES to assist with result analysis.
- Where possible without introducing a delay in the testing pathway, peripheral blood mononuclear cells should be collected and stored for downstream immune function analyses (see Figure 2).
- Analysis for children with a severe COVID-19 presentation, will also include proband only primary immunodeficiency PanelApp gene panel analysis (includes genes associated with immune dysregulation e.g. interferonopathies) for detection of variable penetrance genetic aetiologies, where appropriate.



$If it is not possible to send DNA, then blood/Oragene (saliva) samples may be sent after prior agreement from the Exeter lab.

*In cases where parental samples are unavailable, please contact the Exeter laboratory before sending the patient's sample to discuss whether singleton (patient only) or duo analysis might be possible.

Figure 2 – Summary of the referral pathway for acutely unwell children and young adults (<21 years) with severe COVID-19 presentations

Recruitment to the GenOMICC research study

- All patients on intensive care with COVID-19 are eligible for recruitment to the GenOMICC research study in parallel with diagnostic testing. **A separate sample of EDTA blood** should be submitted to the study.
- The study is a partnership between the GenOMICC consortium and Genomics England and will sequence the genomes of 20,000 people with severe COVID-19 (requiring intensive care) and 15,000 people with mild or no symptoms from the infection.
- The study primarily aims to identify population-level differences between that influence susceptibility to severe COVID-19 and findings from the research study are NOT expected to influence the care of participants during their illness.
- The study is already active in >170 intensive care units across the UK. If you need assistance in identifying your local study coordinator, please contact the central study team at genomicc@roslin.ed.ac.uk
- In the very small number of participants where the research identifies a genomic finding that is likely to explain their severe COVID-19 and that is relevant to their future medical care – for example, a rare genetic immune deficiency – this will be returned to the local Genomic Laboratory Hub for review and diagnostic reporting.
- **Recruitment to the study does NOT replace and should not delay diagnostic testing as results will not be available during the patient's acute illness.**

Supporting Information

https://www.exeterlaboratory.com/test/exome-sequencing-services/

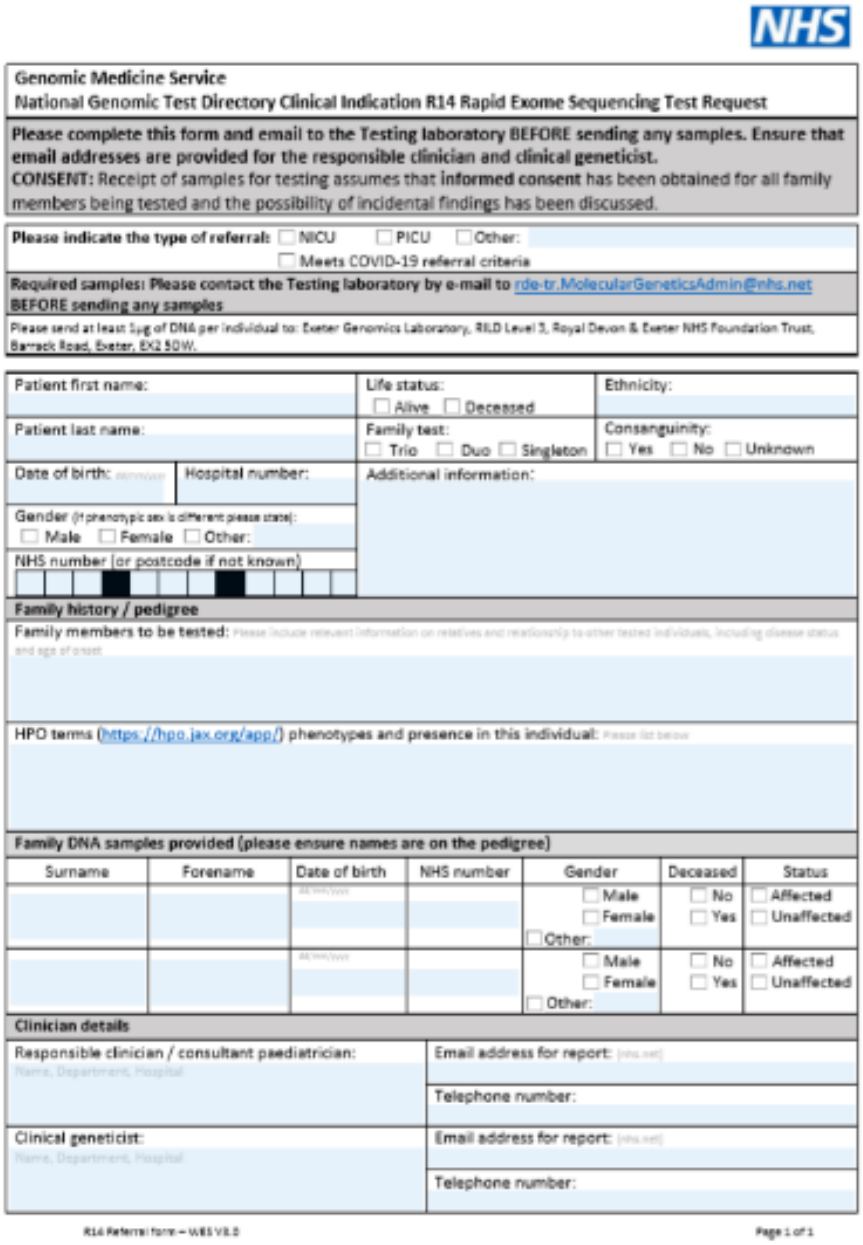
French CE, *et al.* Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children. Intensive Care Med. 2019 Mar 7. doi: 10.1007/s00134-019-05552-x. [Epub ahead of print].

Mestek-Boukhibar *et al*. Rapid Paediatric Sequencing (RaPS): comprehensive real-life workflow for rapid diagnosis of critically ill children. J Med Genet 2018 Nov;55(11):721-728 doi:10.1136/jmedgenet-2018-105396.

Stark Z *et al*. Meeting the challenges of implementing rapid genomic testing in acute pediatric care. Genetics in Medicine. 2018 Mar 15. doi: 10.1038/gim.2018.37

Stals KL *et al*. Diagnosis of lethal or prenatal-onset autosomal recessive disorders by parental exome sequencing. Prenat Diagn. 2018 Jan;38(1):33-43. doi: 10.1002/pd.5175.

Appendix – R14 test referral form



Genomic Medicine Service
National Genomic Test Directory Clinical Indication R14 Rapid Exome Sequencing Test Request

Please complete this form and email to the Testing laboratory BEFORE sending any samples. Ensure that email addresses are provided for the responsible clinician and clinical geneticist.
CONSENT: Receipt of samples for testing assumes that **informed consent** has been obtained for all family members being tested and the possibility of incidental findings has been discussed.

Please indicate the type of referral: ☐ NICU ☐ PICU ☐ Other:
☐ Meets COVID-19 referral criteria

Required samples: Please contact the Testing laboratory by e-mail to rde-tr.MolecularGeneticsAdmin@nhs.net **BEFORE sending any samples**

Please send at least 1µg of DNA per individual to: Exeter Genomics Laboratory, RILD Level 3, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW.

Patient first name:	Life status: ☐ Alive ☐ Deceased	Ethnicity:
Patient last name:	Family test: ☐ Trio ☐ Duo ☐ Singleton	Consanguinity: ☐ Yes ☐ No ☐ Unknown
Date of birth: dd/mm/yyyy / Hospital number:	Additional information:	
Gender (if phenotypic sex is different please state): ☐ Male ☐ Female ☐ Other:		
NHS number (or postcode if not known)		

Family history / pedigree

Family members to be tested: Please include relevant information on relatives and relationship to other tested individuals, including disease status and age of onset

HPO terms (https://hpo.jax.org/app/) phenotypes and presence in this individual: Please list below

Family DNA samples provided (please ensure names are on the pedigree)

Surname	Forename	Date of birth	NHS number	Gender	Deceased	Status
		dd/mm/yyyy		☐ Male ☐ Female ☐ Other:	☐ No ☐ Yes	☐ Affected ☐ Unaffected
		dd/mm/yyyy		☐ Male ☐ Female ☐ Other:	☐ No ☐ Yes	☐ Affected ☐ Unaffected

Clinician details

Responsible clinician / consultant paediatrician: Name, Department, Hospital	Email address for report: (nhs.net)
	Telephone number:
Clinical geneticist: Name, Department, Hospital	Email address for report: (nhs.net)
	Telephone number:

R14 Referral form – WES V3.0 Page 1 of 1