

# **Guidance document:**

## **Rapid Exome Sequencing Service for acutely unwell adults aged 21 to 40 years with COVID-19 with a likely monogenic disorder**

NHS England and NHS Improvement



# **Rapid Exome Sequencing for acutely unwell adults aged 21 to 40 years with COVID-19 with a likely monogenic disorder**

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## Document management

### Revision history

Version	Date	Author	Summary of changes

### Reviewers

This document must be reviewed by the following people:

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## 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.

NHS England have extended the National Genomic Test Directory R14 clinical indication (acutely unwell children with a likely monogenic disorder) to include adults aged 40 years and under with severe COVID-19, thought to be due to a monogenic primary immunodeficiency/immune dysregulation syndrome or another monogenic aetiology.

This Clinical Policy defines expectations of the rapid diagnostic exome sequencing service for these acutely unwell adults with severe COVID-19, which is provided by the network of Genomic Laboratory Hubs (GLHs) as part of the Genomic Medicine Service (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. In recognition that there is a subset of acutely unwell individuals with confirmed or suspected COVID-19, aged 40 years and below thought to have an underlying monogenic aetiology that may explain their severe presentation (e.g. Primary Haemophagocytic lymphohistiocytosis), NHS England have expanded the R14 test eligibility to include this group. To maximise the diagnostic yield, a whole exome sequencing approach is being employed rather than targeted gene panel or clinical exome sequencing. It is important to note that this is a clinical diagnostic test and distinct to any research projects in this field.

## Service Provision

In summary:

- Diagnostic whole exome sequencing (WES) will be performed.
- Cases reviewed by the Testing GLH prior to the Home GLH sending samples, with input from an expert clinical panel where appropriate.
- Cases where testing is likely to provide an immediate change in clinical management for the patient and/or their family.
- Where imminent demise of the patient is predicted then rapid exome sequencing may be performed where results may affect family management.
- 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.
- Trio testing where possible, with duos or singletons accepted according to sample availability.
- 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 adults ≤40 years with COVID-19 with no predisposing medical conditions/risk factors, thought to have an underlying monogenic aetiology that may explain their severe presentation (see below for more details)	Following discussion with Clinical Genetics, the patient's local management team and the Testing laboratory.  All test requests should be emailed to the Exeter laboratory before sending samples and consenting the family, to confirm eligibility for the test.	Clinical Genetics. Clinical Immunology

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

- Adults ≤40 years of age on ICU with confirmed/suspected severe COVID-19 infection and another risk factor for monogenic primary immunodeficiency/immune dysregulation syndrome or other monogenic aetiology (such as syndromic features; personal or family history of severe infection or early onset severe autoimmunity) and in the absence of known co morbidities' in the absence of known co-morbidities' (Diabetes Mellitus, significant pre-existing lung disease, renal disease, ischaemic heart disease or liver disease) or immunosuppressive/immunomodulating medications (azathioprine, mycophenolate, ciclosporin, tacrolimus, sirolimus, JAK inhibitors and monoclonal antibodies) . In the absence of any such clinical indicators, the patient is unlikely to have a monogenic cause underlying their severe presentation and recruitment to the GenOMICC study would be more appropriate than R14 testing (see below).
- There are a subset of immunodeficiency/immune dysregulation disorders (e.g. primary HLH - Hemophagocytic Lymphohistioytosis), that may be difficult to discriminate from Paediatric Multisystem Inflammatory Syndrome temporally associated with COVID-19 (PIMS-TS) that can affect young adults after COVID-19 infection. Clinical judgement will be required to determine the patients for whom this test may be appropriate, but where the clinical presentation and blood markers are entirely consistent with PIMS-TS, then recruitment to the GenOMICC study would be more appropriate than R14 diagnostic exome sequencing.
- Discussion with an expert in the appropriate clinical area pertinent to the patient's clinical presentation is advised, prior to referral (e.g. Immunologist). Evidence of a positive COVID-19 viral RNA test is not an eligibility requirement.
- Where possible and without introducing a delay in the testing pathway, peripheral blood mononuclear cells (PBMCs) should be collected and stored for downstream immune function analyses (see Figure 1 and Analysis section). This may be required after the acute illness episode.

## Testing Pathways

### Summary

Based on current service provision and previous translational experience, the Royal Devon & Exeter NHS Foundation Trust, Exeter (South West GLH) has been assigned this service.

### Role of the ICU team

The ICU team, Immunologist 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 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. The referral must be checked to indicate that the individual is thought to be affected by COVID-19.

### 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
- 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 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

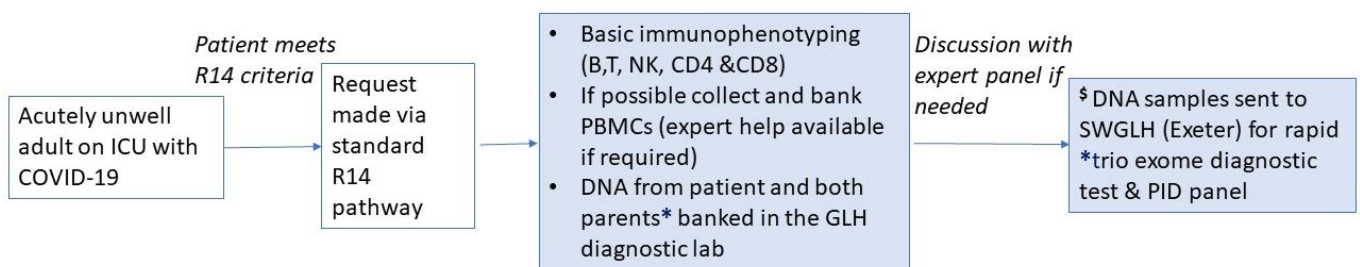


Figure 1 – Summary of the role of the Home GLH

## 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 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 ICU team if required.
- Participation in Management Information data collection.

## Analysis

- Where a trio has been sequenced, analysis for ICU 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
- Mitochondrial DNA variants are analysed but large-scale rearrangements and low levels of heteroplasmy are not detectable by exome sequencing. Testing should be requested from a specialist laboratory if a mitochondrial disorder is suspected.

## 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	21
Testing GLH activity 2	All samples and required data received from Home GLH	Issue of final report for probands with confirmed clinically relevant finding	28

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 ICU teams to support implementation of the service and can help advise on the likelihood of a monogenic disorder alongside local Immunology and Infectious Disease clinicians 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 ICU teams, Immunologists, Infectious Disease Specialists, Clinical Geneticists and Clinical Scientists will be required for interpretation and reporting of complex cases.

## R14 – rapid sequencing considerations

### Consent

The points below should be discussed (in person/by telephone) by the Clinical team with the family members being sequenced, prior to sequencing. Patients on ICU may be too unwell to give informed consent, in these cases the test request should be discussed with the family members and timing of the test driven by the patient's clinical needs and likely impact on acute management.

- 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 date may reveal the causative mutation.

### 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](mailto: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/>

# Appendix – R14 test referral form



<b>Genomic Medicine Service</b> <b>National Genomic Test Directory Clinical Indication R14 Rapid Exome Sequencing Test Request</b>																		
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. <b>CONSENT:</b> 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: <input type="checkbox"/> NICU <input type="checkbox"/> PICU <input type="checkbox"/> Other: _____ <input type="checkbox"/> Meets COVID-19 referral criteria																		
Required samples: Please contact the Testing laboratory by e-mail to <a href="mailto:rde-tr.MolecularGeneticsAdmin@nhs.net">rde-tr.MolecularGeneticsAdmin@nhs.net</a> BEFORE sending any samples																		
Please send at least 5µg 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:		Ethnicity:														
		<input type="checkbox"/> Alive <input type="checkbox"/> Deceased																
Patient last name:		Family test:		Consanguinity:														
		<input type="checkbox"/> Trio <input type="checkbox"/> Duo <input type="checkbox"/> Singleton		<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown														
Date of birth: <small>(dd/mm/yyyy)</small>	Hospital number:		Additional information:															
Gender (if phenotypic sex is different please state):																		
<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other: _____																		
NHS number (or postcode if not known)																		
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<b>Family history / pedigree</b>																		
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 ( <a href="https://hpo.jax.org/app/">https://hpo.jax.org/app/</a> ) 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												
		<small>(dd/mm/yyyy)</small>		<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other: _____	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> Affected <input type="checkbox"/> Unaffected												
		<small>(dd/mm/yyyy)</small>		<input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Other: _____	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> Affected <input type="checkbox"/> Unaffected												
<b>Clinician details</b>																		
Responsible clinician / consultant paediatrician:				Email address for report: <small>(nhs.net)</small>														
<small>Name, Department, Hospital</small>				Telephone number:														
Clinical geneticist:				Email address for report: <small>(nhs.net)</small>														
<small>Name, Department, Hospital</small>				Telephone number:														