East Midlands Neonatal
Operational Delivery Network

NETWORK GUIDELINE

Guideline:	**Rapid R14 Whole Exome Sequencing Pathway for Critically Ill Neonates**
Version:	**1**
Date:	**November 2021**
Review Date:	**November 2024**
Approval:	**EMNODN Clinical Governance Group**
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Consultation:	**Regional Clinical Genetics Services and EMNODN Clinical Governance Group**
Distribution:	**Neonatal Units within the EMNODN**
Risk Managed:	**Care of babies with likely monogenic disorders, communication of results.**

This document is a guideline. Its interpretation and application remains the responsibility of the individual clinician, particularly in view of its applicability across the different Trusts in the East Midlands Neonatal Operational Delivery Network. Please also consult any local policy/guideline document where appropriate and if in doubt contact a senior colleague.

Caution is advised when using guidelines after a review date.

REVIEW AND AMENDMENT LOG

Version	Type of Change	Date	Description of Change
1	-	Nov 2021	New Guideline

Key points:

- The rapid-WES service is intended for acutely unwell neonates/children with:
    - Features highly suggestive of a monogenic disorder, for whom;
    - A genetic diagnosis could provide an immediate change in management
- Results will usually be communicated within 2-3 weeks of sample receipt
- Testing the parents at the same time as the child (trio analysis) facilitates detection of de novo, recessive and X-linked variants and provides a faster diagnosis
- This testing should never be discussed with parents prior to consulting the local genetics consultant AND before agreement with the lead team in Exeter
- Form R14 “Rapid Exome Sequencing Request Form” should be completed by/with the neonatal/paediatric consultant or Clinical Geneticist
- **A Record of Discussion form should be completed with the family (Consent form)**
- If any delay is encountered during the referral process, it is acceptable to send 0.5-1ml blood (EDTA bottle) from the patient to Molecular Genetics for DNA storage
- Results should not be discussed with the family until a formal report is issued. **Provisional e-mails sent to a clinician do not constitute a final diagnosis.** Clinical collaboration will be organised and only then, a final clinical report be issued.
- In the event of transfer both the receiving team and clinical genetics team involved must be informed of a rapid WES in progress.

Abbreviations used in this document:

GLH	Genomic Laboratory Hub
NHSE	NHS England
NICU	Neonatal Intensive Care Unit
VUS	Variant of uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing

Contents

1. Background Information

10-15% of infants are admitted to NICU in some capacity, thus individual rare diseases (less than 1:2000 population) are collectively common in this cohort. Factors complicating diagnosis of rare disorders are the facts that full clinical phenotypes may not be manifest in neonates, and genetic heterogeneity can be immense.

As of 01 October 2019 **a new service model for Rapid WES (Whole Exome Sequencing) has been introduced through England's regional Genomic Laboratory Hubs (GLHs).** The service is designed to optimise our patients' access to a unified, rapid, diagnostic service.

The ultimate goal of rapid sequencing in acutely ill neonates is to alter outcomes in such a way as to provide thoughtful, effective care and management to this vulnerable population. Among those tested to date, acute management decisions have been altered for 13% of infants and there is evidence to support some improved outcomes and overall reduction in healthcare costs for such early molecular diagnosis (French *et al.*, 2019).

The rapid WES utilises a gene-agnostic trio approach designed to identify de novo, autosomal recessive and X-linked monogenic disorders.

1.1 Monogenic disorders and diagnosis

Monogenic diseases are conditions causally related to genomic changes, or variants, in a single gene (often referred to as SNV's). This collection of diseases is currently most amenable to diagnosis through WGS/WES because the causative variants frequently involve a small number of DNA nucleotides, in one or a handful of genes. This test is not designed to detect larger copy number variants (CNV's).

WES describes analysis of protein-coding regions of DNA (exons) which account for approximately 2% of the whole human genome. More than 85% of disease causing variants are within these exons. The test therefore has a high diagnostic yield and examples of diagnosis by this method have facilitated specific changes in treatment or management for NICU patients. Specific changes to management enabled by these rapid results have included institution of palliative care, initiation of new subspecialist involvement, changes in medication, diet and directed choice of imaging technique. Of diagnoses made prior to discharge or death in some studies, 85% were considered to have acute clinical utility (Petrikin *et al.*, 2015).

1.2 A non-exhaustive list of potential candidates for Rapid WES testing

System	Presentation / Clinical phenotype
Neurological	Infantile encephalopathy / epilepsy
	Progressive neuromuscular disorder
Renal	Nephrotic syndrome
Metabolic	SOME specific disorders
Endocrine	Suspected neonatal diabetes
Respiratory	Primary ciliary dyskinesia
Specific syndromes	Only where molecular diagnosis would help guide immediate clinical care e.g. Genitopatellar syndrome

2. Pathway Guidance

Below is a summary of the pathway required for patient selection and referral for a rapid WES. Details regarding specific centres and the required pathway are below in section 2.4 with summary flowcharts found in **Appendix 2.**

2.1 Selection of appropriate case candidates

The rapid-WES service is intended for acutely unwell neonates/children with features highly suggestive of a monogenic disorder, for whom a genetic diagnosis could provide an immediate change in clinical management. NHSE funds 700 tests per year across England.

This pathway **does not** encompass the following instances:

- Excluding a genetic diagnosis when a non-genetic cause is highly likely to explain the clinical presentation e.g. Hypoxic-Ischaemic Encephalopathy, Extreme prematurity, Infection
- Fetal genomic evaluation or antenatal concerns related to prior pregnancies
- Post mortem or immediate peri-mortem investigations
- Genetic concerns other than monogenic disorders whose rapid diagnosis may be directly relevant to clinical care
- Where there is unlikely to be an immediate direct change in management. (Other pathways for genome sequencing may be more appropriate).

WES is also not appropriate where a different route of testing would be more suited to the monogenic disorder suspected e.g. Cystic Fibrosis, Mucopolysaccharidosis suggested on biochemical testing. Only nuclear mitochondrial disorders are detected through WES. Therefore, if a mitochondrial disorder is suspected, further wider discussion is likely to be required.

Expansion repeats and uniparental disomy are not routinely detected by WES. Therefore for infants in which a differential diagnosis includes genetic conditions with this causation direct testing should also be performed e.g. Prader-Willi or myotonic dystrophy.

Other limitations include intronic variants, structural variants (e.g. translocations and inversions) and variants in difficult regions such as those with pseudogenes. This means that other conditions such as Spinal muscular atrophy (SMA) are difficult to diagnose through this testing approach.

Due to these limitations it may be appropriate to perform parallel testing e.g. WES alongside SMA and myotonic dystrophy testing.

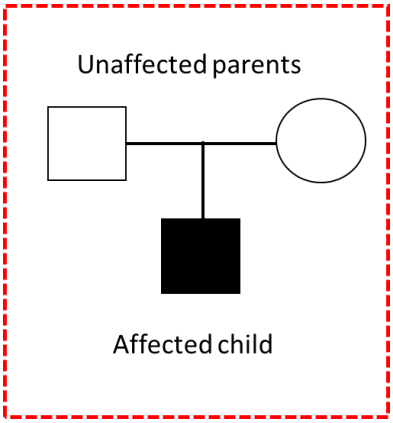
Microarray CGH testing or urgent karyotypes should continue to be requested in accordance with normal practice if a chromosome disorder is suspected.

If rapid diagnosis *is not likely to impact immediately on clinical management*, then testing delayed as an outpatient may be more appropriate and WES **should not** be requested e.g. dysmorphic features or congenital anomalies without acute management implications or when testing is for future family planning.

2.2 Trio Testing

For the WES service, a “gene-agnostic trio approach” is used (Figure 1).

Figure 1: Trio Exome Testing identifies de novo, or inherited (autosomal recessive, X-linked) variants by testing the parents at the same time as the child, and provides a faster diagnosis.



If only one parent (or neither) is available for testing, the analysis will be based on virtual gene panels selected by patient's phenotype (chosen from PanelApp and agreed with the clinical team when the test is requested https://panelapp.genomicsengland.co.uk/panels/)

***Remember, trio testing will reveal non-paternity.** Donor gametes through IVF conception should also be declared within the referral process. Counselling for these matters is discussed within consent below.*

2.3 Referral and requesting process

The lead team for WES testing in England is based in Exeter. **Testing should never be discussed with parents prior to consulting the lead team in Exeter.**

Some cases appropriate for WES testing would already fall within existing local clinical practice pathways, and be referred to clinical genetics at the outset. Existing ward consults may be subsequently considered appropriate for WES and will follow this pathway. There may be a need for more detailed and comprehensive information regarding these patients at the point of primary referral to genetics, to facilitate a smoother and faster WES pathway.

There will be a cohort of patients, though likely a small number, who would not previously have been referred to genetics, but who may still be appropriate for the WES pathway. These could include seemingly isolated neurological deficits, metabolic presentations or endocrine abnormalities for instance. After early discussion with the relevant sub-specialist consultant (local or regional), where the possibility of a monogenic disorder arises, **an additional referral to the on-call consultant for clinical genetics** should be made PROMPTLY.

If any delay is encountered during the referral process, it is recommended to send 0.5-1ml blood (EDTA bottle) from the patient to Molecular Genetics for DNA storage.

Below is an outline of the process. Please see specific pathways below for each hospital within the EMNODN.

After discussion with genetics, but BEFORE discussing the test with the parents form R14 "Rapid Exome Sequencing Request Form" (editable PDF) should be completed by/with the neonatal/paediatric consultant or clinical geneticist. An up-to-date form is located on the Exeter Laboratory website (http://www.exeterlaboratory.com/test/exome-sequencing-services/).

The form should be emailed to the Exeter genomics laboratory (email address on the form). See local pathways for further individuals to be copied in. As much relevant clinical information as possible should be included in the referral from.

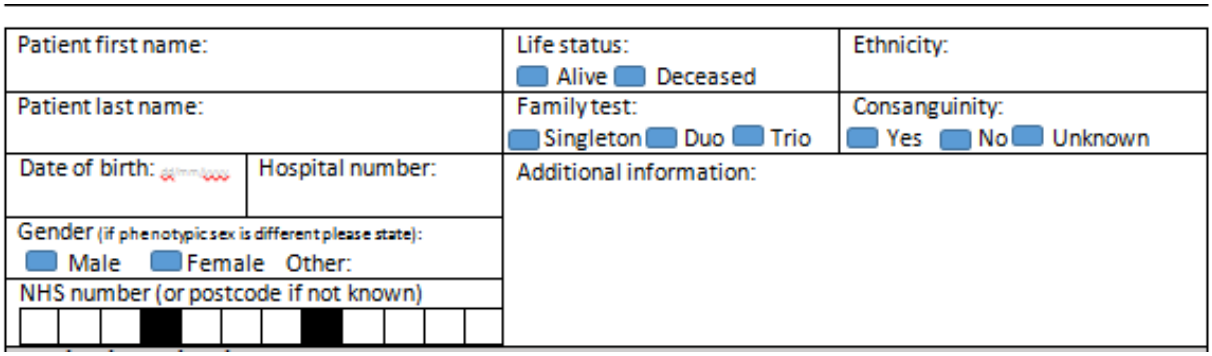
1. The Exeter GLH will triage the referral and confirm suitability for WES testing. They may telephone or email the referrer for further information or clarification. Once approved, the testing can be subsequently discussed with the parent/s. A record of discussion form (consent form) should be completed and filed in the medical notes. An up-to-date form is

located on the Exeter Laboratory website (http://www.exeterlaboratory.com/test/exome-sequencing-services/).

2. After the consent process has been undertaken, the **request form AND blood samples must be sent together to the LOCAL molecular genetics laboratory,** not directly to Exeter. Wherever possible, as described in trio testing, samples from both parents and the infant should be sent to the laboratory. If this is not possible on the same day, or at all, please discuss this directly by phone with the laboratory.

2.3a Clinical information for referral

Demographic and basic family information should include whether the family test is “Singleton” (without biological parent samples), “Duo”(with one parent’s sample), or “Trio” (with samples from both biological parents).



Information should include both **details of the child’s clinical presentation** on which a monogenic disorder is suspected**, and any family history** as this may be essential for interpretation of the DNA sequence data and/or selection of appropriate gene panels to use where trio testing samples are not possible. The **family structure should be included** (see below). Phenotype (or “HPO”) terms should be used to describe abnormalities, or descriptive text can be used. The HPO terms can be found via a link on the referral forms.

Additional information should include differential diagnoses under consideration, results of relevant investigations to date (or pending) e.g. neuroimaging, muscle biopsy, and any previous genetic investigations. It is important to include as much clinical information as possible as this will give the best chance of a diagnosis from the test. The form must be filled out electronically and the editable PDF forwarded not a scanned copy.

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

2.3b Consent, Counselling & Parental expectations

WES will not always lead to certainty, and may introduce new uncertainties (Newson, 2017). Comprehensive consenting is required and it may be appropriate for these discussions to be held jointly with both the neonatal/paediatric and clinical genetic teams. We will refine and evolve the supporting documents and information sources for this process as it becomes embedded in practice.

Counselling

It is key for parents to have an opportunity to make a well informed and carefully considered decision regarding genomic testing for their baby. As with previous genetic tests, the principle that this counselling be non-directive should be respected (Wilkinson *et al.*, 2016). This is likely to be a time consuming process it may be appropriate for this to be a joint venture between the Consultant Neonatologist/Paediatrician and Consultant Clinical Geneticist or Genetic Counsellor

Pre-test counselling specifics

As a minimum, pre-test counselling should address the range of information that would be relevant to parents' and doctors' decisions about treatment. **The possible outcomes of testing are:** diagnostic, negative, or uncertain significance (Variant of uncertain significance – VUS). Counselling should specifically explore the possibility of identifying VUS (Wilkinson *et al.*, 2016). Testing may have other consequences for the child in future, and the wider family. This may lead to subsequent familial referrals to genetics.

Specifically exploring **the biological heritage for this baby ie, donor eggs and/or sperm** is important prior to proposing trio testing. Testing of this nature will reveal the biological relationships between parents and child ie, non-paternity will be identified.

The lab must also be made aware if a biological parent has received an allogeneic bone marrow or stem cell transplant.

Testing evolves and it is possible that the sample/s can be re-tested in the future for further investigations.

Points of concern

Ethical issues raised are complex, but similar to those for investigations already offered to patients in NICU.

- **False positives**
  There is a possibility that a mutation reported as causative at this point in time will be reassessed in the future and found to be non-pathogenic.-

- **Incidental findings**
  Information pertaining to adult-onset or unrelated illnesses are not specifically analysed in this process. It is unlikely that such incidental findings will be detected, therefore the risk of causing psychological harm is low. It is, however, important as a family must not incorrectly take the impression that their child "has no genetic problems" or "no abnormal genes" as a result of negative WES testing. Equally they do need to be aware of the possibility of an incidental finding being discussed.

- **Variants of unknown significance**
  VUS are sequences which are not common in the general population but we can't be clear a pathogenic link exists. With time some will be clarified as pathogenic, whilst others will be ascertained as non-pathogenic. Some will remain unclear; counselling for WES testing should include this element carefully (Petrikin *et al.*, 2015).

- **Private and/or commercial testing**
  Some parents express a desire for this kind of information and might be able to access commercial whole genome sequencing in the future (Nuffield Council on Bioethics, 2018) Several companies offer, or are planning to offer, newborn screening tests that search for large numbers of genetic conditions.

2.4 Local pathways

The following hospitals Trusts have clinical genetic services on site:
Nottingham University Hospitals NHS Trust
University Hospitals of Leicester NHS Trust
Northampton General Hospital NHS Trust*

*The clinical genetics team in Northampton serve the population of Northamptonshire but are part of the Leicester, Rutland and Northampton Regional Genomics service.

For other neonatal units please see the table below for your local clinical genetics service.

Neonatal Unit	**Clinical Genetics Service**
Pilgrim Hospital, Boston	Nottingham
Royal Derby Hospital	Nottingham
Lincoln County Hospital	Nottingham
King's Mill Hospital, Mansfield	Nottingham
Kettering General Hospital	Northampton
Queen's Hospital, Burton	West Midlands Regional Genetics Service

Each clinical genetics service has personalised pathways for requesting an R14 rapid WES but the principles are as described above.

Each regional pathway (Leicester, Northampton and Nottingham,) can be found below in Appendix 2.

If a baby is transferred to another unit (within or out of region) it is essential the receiving team are informed a rapid WES is in progress and the clinical genetics team involved informed. This prevents delays in the communication of results, both to the clinical team and parents and potential delays in treatment.

3. Results and Outcomes

3.1 Receiving & actioning preliminary findings & results

If no diagnosis is found, the report will be issued, usually by email, within 2-3 weeks of the samples being received.

If a possible, or likely, diagnosis is found the consultant named on the referral paperwork/request will be contacted, again by email, with details of the possible findings. **This stage must NOT be used as information for the family** and does not constitute a final diagnosis. Further discussion and exploration of whether the finding fits with the existing clinical picture may be required, and in some cases an MDT may be convened.

Only then, will a clinical report be issued – this will be by email. This will be supported by a genomic laboratory report. Discussions should follow between clinical genetics and the local clinician to explore who, where and how information giving might best be facilitated with the family.

4. Training

All middle grade and senior neonatal practitioners should be aware of the contents of this guidance. Neonatal, Paediatric consultants, and senior trainees for their interest, can access further information and support around counselling and consent from the clinical genetics teams. Online training resources are also available.

A Genomics Education Programme has been set up to ensure staff in healthcare have the knowledge, skills and experience to deliver genomic and precision medicine. This includes an introduction to genomics and bioinformatics: https://www.genomicseducation.hee.nhs.uk/ Additional specific training resources and materials will be developed in parallel with the evolution of this NHS England service.

5. References

Genomics England; Department of Health & Social Care, https://www.genomicsengland.co.uk/

Anderson, J. A. *et al.* (2017) 'Parents perspectives on whole genome sequencing for their children: Qualified enthusiasm?', *Journal of Medical Ethics*. doi: 10.1136/medethics-2016-103564.

French, C. E. *et al.* (2019) 'Whole genome sequencing reveals that genetic conditions are frequent in intensively ill children', *Intensive Care Medicine*. doi: 10.1007/s00134-019-05552-x.

Newson, A. J. (2017) 'Whole genome sequencing in children: Ethics, choice and deliberation', *Journal of Medical Ethics*. doi: 10.1136/medethics-2016-103943.

Nuffield Council on Bioethics (2018) 'Whole Genome Sequencing in Babies'. Available at:

http://nuffieldbioethics.org/wp-content/uploads/Nuffield-Council-on-Bioethics-briefing-note-whole-genome-sequencing-of-babies.pdf.

Petrikin, J. E. *et al.* (2015) 'Rapid whole genome sequencing and precision neonatology', *Seminars in Perinatology*. doi: 10.1053/j.semperi.2015.09.009.

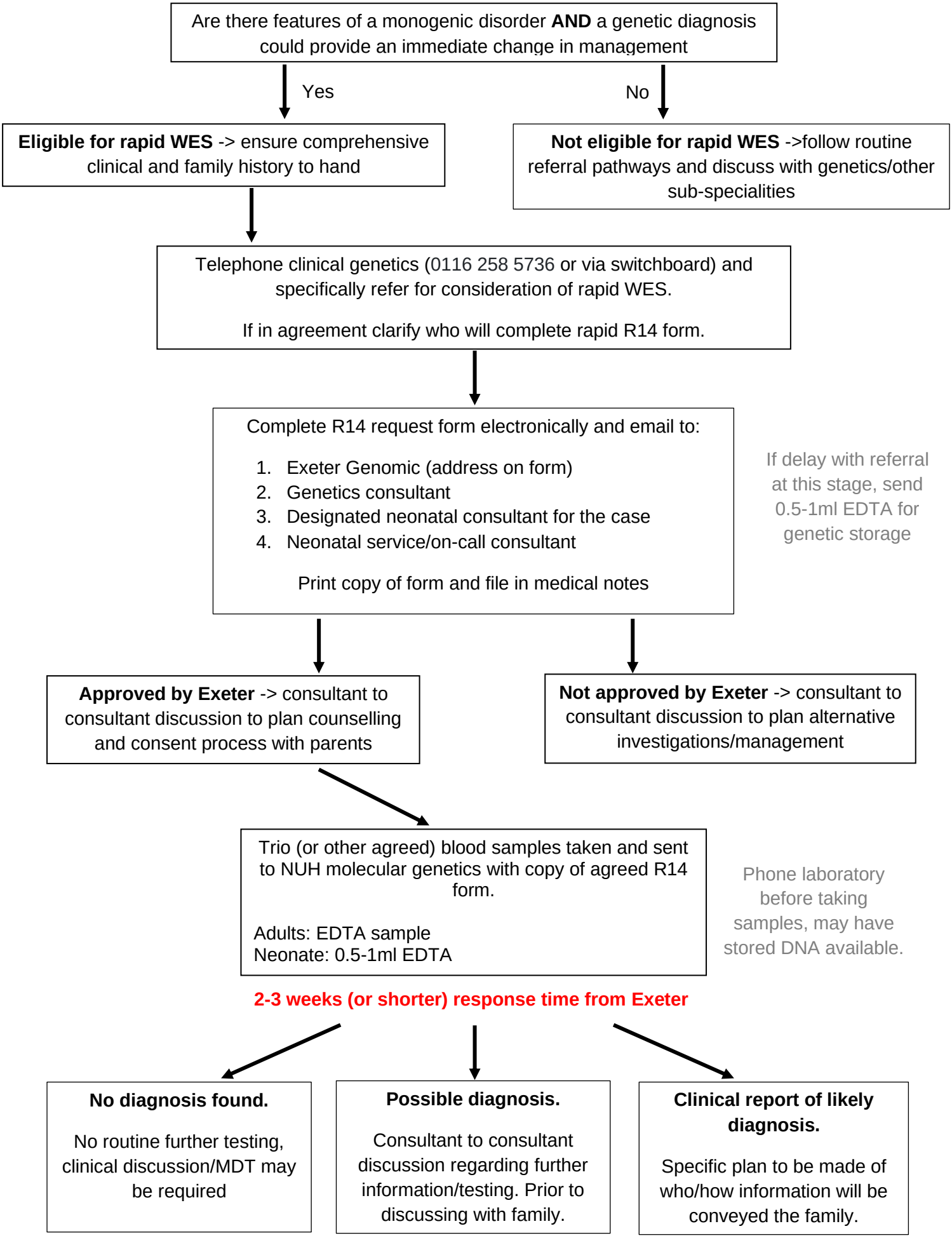
Smith, L. D., Willig, L. K. and Kingsmore, S. F. (2016) 'Whole-exome sequencing and whole-genome sequencing in critically ill neonates suspected to have single-gene disorders', *Cold Spring Harbor Perspectives in Medicine*. doi: 10.1101/cshperspect.a023168.

Wilkinson, D. J. C. *et al.* (2016) 'Genomic intensive care: Should we perform genome testing in critically ill newborns?', *Archives of Disease in Childhood: Fetal and Neonatal Edition*. doi: 10.1136/archdischild-2015-308568.

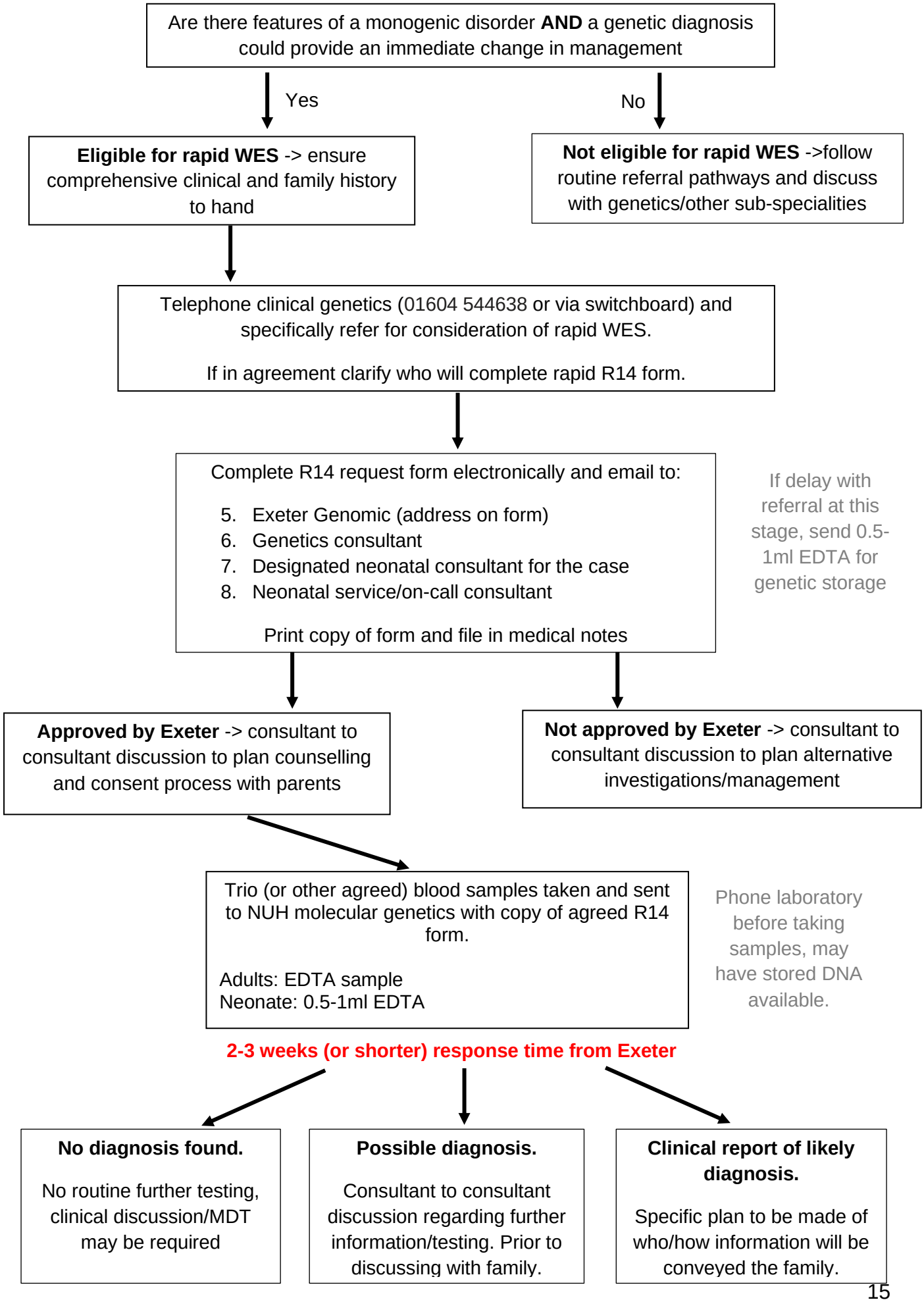
Appendix 1

Record of discussion form (requires printing) and editable PDF R14 request form; www.exeterlaboratory.com/test/exome-sequencing-services/

Appendix 2
Flow Chart of Rapid WES Pathway for Leicester Clinical Genetics Service



Flow chart of rapid WES pathway for Northampton



Flow chart of rapid WES pathway for units served by Nottingham Clinical Genetics Service

Are there features of a monogenic disorder **AND** a genetic diagnosis could provide an immediate change in management

Yes

No

Eligible for rapid WES -> ensure comprehensive clinical and family history to hand

Not eligible for rapid WES ->follow routine referral pathways and discuss with genetics/other sub-specialties

Telephone on call consultant for clinical genetics (0115 924 9924 ext 59480 or via switchboard) and specifically refer for consideration of rapid WES.

Complete R14 request form electronically and email to:

- Exeter Genomic lab (address on form)
- Genetics on-call consultant
- NUHNT.clinicalgenetics@nhs.net
- Designated neonatal/paediatric consultant for the caseNeonatal/paediatric service/on-call consultant

Print copy of form and file in medical notes

If delay with referral at this stage, send 0.5-1ml EDTA for genetic storage

Approved by Exeter -> consultant to consultant discussion to plan counselling and consent process with parents
Please document “approved” on the form in the notes

Not approved by Exeter -> consultant to consultant discussion to plan alternative investigations/management
Please document “not- approved” on the form in the notes

Trio (or other agreed) blood samples taken and sent to NUH molecular genetics with copy of agreed R14 form.

Adults: EDTA sample
Neonate: 0.5-1ml EDTA

If necessary, phone laboratory before taking samples, may have stored DNA

2-3 weeks (or shorter) response time from Exeter

No diagnosis found.

Clinical discussion/MDT may be required to decide if any further testing indicated

Possible diagnosis.

Consultant to consultant discussion regarding further information/testing. Prior to discussing with family.

Clinical report of likely diagnosis.

Specific plan to be made of who/how information will be conveyed the family.