Schedule of Accreditation

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Newcastle upon Tyne Hospitals NHS Foundation Trust

Issue No: 004 **Issue date:** 07 July 2025

Newcastle NHS Highly Specialised Service for Rare Mitochondrial Disorders
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Testing performed at the above address only

Laboratory location:

Location details		Activity
Address NHS Highly Specialised Service for Rare Mitochondrial Disorders 4th Floor Cookson Building The Medical School Newcastle University Framlington Place Newcastle upon Tyne NE2 4HH	**Contact** Amritjit Singh (Contact details as above)	Multidisciplinary - Mitochondrial Disorders (Mitochondrial Genetics, Biochemistry, Histopathology)

DETAIL OF ACCREDITATION

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE	<u>Mitochondrial Disorders</u>	Using In-house documented methods incorporating manufacturer's instruction where relevant.
	<u>Enzyme histochemistry/histology</u>	
Human tissue – skeletal and cardiac muscle (including but not limited to list below) I.V. Septum Right Ventricle Left Ventricle Right Atrium Left Atrium Liver Adrenal Kidney Diaphragm muscle Psoas muscle Quadriceps muscle Triceps Gastrocnemius Biceps Paraspinal muscle Tibialis Anterior Prostate Large Intestine	Preparation of frozen tissue sections for histopathological and histochemical assessment of mitochondrial function	Sectioning frozen material using the cryostat MDS 127 Sample preparation for histochemistry MDS 22
	Staining of prepared tissue for the purposes of histological and enzyme histochemical analysis to aid diagnosis of mitochondrial genetic disease.	Using In house procedures: Haematoxylin and Eosin stain MDS121 Cytochrome *c* oxidase MDS120 Succinate dehydrogenase MDS128 Sequential Cytochrome *c* oxidase-Succinate dehydrogenase MDS119 Modified Gomori Trichrome stainMDS123 And: Bright OTF 5000 Cryostat MDS446 Zeiss Axiovision Image Acquisition System MDS464

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	Mitochondrial Disorders (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
	Respiratory Chain enzyme measurements Biochemical measurement of mitochondrial respiratory chain enzyme activities for (including combinations):	
Mitochondrial fractions from skeletal or cardiac muscle,fibroblasts and myoblasts	Citrate synthase Respiratory chain complex I Respiratory chain complex II Respiratory chain complex III Respiratory chain complex IV	The complex enzyme activity is calculated based on the measurement of the absorbance change during the biochemistry oxidoreductase reaction, then the activity is expressed as the ratio by dividing with citrate synthase activity Using In house procedures: Preparation of Mitochondrial Fractions – fibroblasts and myoblasts MDS91 Preparation of Mitochondrial Fractions – skeletal or cardiac muscle MDS94 Preparation of Mitochondrial Fractions – pig heart muscle MDS93 Biochemical assay of Citrate Synthase MDS80 Biochemical assay of respiratory chain complex I activity MDS82 Biochemical assay of respiratory chain complex II activity MDS86 Biochemical assay of respiratory chain complex III MDS88 Biochemical assay of respiratory chain IV complex MDS90

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	<u>Mitochondrial Disorders</u> (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
Mitochondrial fractions from skeletal or cardiac muscle and fibroblasts (cont'd)		And: Sorvall Lynx 4000 centrifuge MDS487 Cary Spectrophotometers and circulators ID MDS486
	<u>Quadruple Immunofluorescence</u>	
Human tissue – skeletal and cardiac muscle	Quantitative measurements of key OXPHOS protein abundance in individual muscle fibres within a 10µm tissue section, relating findings to a mitochondrial mass marker.	Quadruple Immunofluorescence of Mitochondrial Respiratory Chain Complex I and Complex IV in Human Muscle Tissue Using inhouse procedures: Guidelines for medical trainees using the immunofluorescence microscope MDS129 Quadruple analysis online software tool MDS125 Quadruple Immunofluorescence respiratory chain complexes MDS126 And: Zeiss Axiovision Image Acquisition System MDS464 Mat Lab (computer analysing system, MathWorks®) Axio Cam MRC-ZEIZZ Microscope MDS508

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	Mitochondrial Disorders (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
	Genomics analysis for the purpose of clinical diagnosis of mitochondrial disorders.	Manual and automated DNA/RNA extraction and quantification
Blastomeres and cytoplasts	Mitochondrial, genomic and nuclear DNA and RNA extraction, quantification and quality check for subsequent in-house analysis (see below), referral to specialist centres and long-term storage Preparative pre-examination steps listed first	PGD- Blastomere lysis and pyrosequencing PCR (MDS215) and Mitochondrial Donation - Cytoplast lysis and pyrosequencing PCR (MDS893) Individual muscle fibres: Microdissection and lysis of single muscle fibres (MDS192)
Peripheral Blood Cord Blood		Extraction of DNA from blood using EZ1 Advanced Workstation (MDS130)
Fresh or frozen human tissue, fibroblasts, buccal and nuclear pellets		Extraction of DNA from tissue using EZ1 Advanced XL Workstation (MDS850)
Urine		Extraction of DNA from urine pellet using EZ1 Advanced Workstation (MDS133) DNA Quantification for QC purposes using: Nanodrop 1000 - measuring nucleic acids (MDS451) Qubit MDS216

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	Mitochondrial Disorders (cont'd) Genomics analysis for the purpose of clinical diagnosis of mitochondrial disorders. (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
Fibroblasts		RNA extraction: Manual extraction using Promega Reliaprep RNA Cell Miniprep System MDS140 and MDS141 RNA Quantification for QC purposes using: Nanodrop 1000 - measuring nucleic acids (MDS451) Spectrophotometer MDS922, MDS919, MDS921, MDS923
RNA extracted from Fibroblasts	Generation of cDNA by reverse transcriptase for subsequent in-house analysis (see below)	cDNA generation Manual method using reverse transcription MDS418
Mitochondrial and nuclear DNA and RNA extracted in-house from the sample types listed above or received from external sources	Detection of known or unknown sequence variants – SNVs, indels, and splicing variants	Sanger Sequencing Standard primer design methodology (MDS 195 and MDS196), and PCR amplification. Sanger Sequencing performed using Applied Biosystems ABI 3500xl Capillary Electrophoresis analyser (MDSMDS163, MDS175, MDS180, MDS189,MDS863) Analysis: mtDNA analysis using SeqScape software; Applied Biosystems MDS 213

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	<u>Mitochondrial Disorders</u> (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
	Molecular Genetics	nDNA analysis using Mutation Surveyor software; SoftGenetics MDS214 Clinical interpretation MDS208
		<u>Fragment Size Analysis</u>
cDNA	Analysis of potential splicing variants	RNA Analysis of potential splice site variants MDS418 Sanger Sequencing performed using Applied Biosystems ABI 3500xl Capillary Electrophoresis analyser (MDSMDS163, MDS175, MDS180, MDS189,MDS863) Analysis using Mutation Surveyor software; SoftGenetics MDS214
Mitochondrial DNA extracted in-house from the sample types listed above or received from external sources	Detection of Large-scale mtDNA rearrangements	Long Range PCR with size analysis : Using: Three separate long-range PCR assays with resolution by gel electrophoresis and imaging by BioRad ChemiDoc (MDS147 and MDS457) Heteroplasmy assessment of mtDNA rearrangement MDS157 Breakpoint mapping of mtDNA rearrangement MDS233, MDS147, MDS230

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	Mitochondrial Disorders (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant.
Mitochondrial and nuclear DNA extracted in-house from the sample types listed above or received from external sources	Gene screening of large gene panels and mtDNA for genetic variants	Next Generation Sequencing: Library Preparation Method: Manual set up: Ion Xpress Plus Fragment Library Kit (MDS223) IonLibrary Taqman Quantitation Kit (MDS226) PCR using thermal cycler, qPCR (Instrument), Agilent2100 Bioanalyser and Qubit (IMDS216), Sequencing on Ion Torrent S5 Interpretation of sequence variants with preparation using Ion Chef (MDS208) Next generation Sequencing mtDNA Analysis (MDS233)
Nuclear DNA	SNVs, small indels	Qubit (MDS343) Data analysis using VARDB (MDS234) Variant interpretation (MDS208)

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
HUMAN BODY TISSUE (cont'd)	Mitochondrial Disorders (cont'd) Genomics analysis for the purpose of clinical diagnosis of mitochondrial disorders.	Using In-house documented methods incorporating manufacturer's instruction where relevant.
Mitochondrial and nuclear extracted in-house from the sample types listed above or received from external sources	Detection of known SNVs and indels	Pyrosequencing Using: Qiagen Pyromark Q24 Pyrosequencer, PCR amplification using in-house designed primers and Pyromark Analysis using Pyromark Q24 software; Qiagen. (MDS197, MDS202, MDS200 and MDS201)
Mitochondrial DNA in-house from the sample types listed above or received from external sources	mtDNA depletion	Real Time PCR Using Applied Biosystems StepOne Plus real-time PCR system (MDS159) and in house procedures for detecting mitochondrial DNA depletion (MDS159)
	mtDNA Deletions	Real Time PCR Using Applied Biosystems StepOne Plus real-time PCR system (MDS159) and in house procedures for detecting mitochondrial DNA deletions in muscle fibres, single cells and tissue homogenate (MDS 157)
END		