Schedule of Accreditation

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9027 **Accredited to ISO 15189:2012**	**The Newcastle upon Tyne Hospitals NHS Foundation Trust** **Issue No:** 002 **Issue date:** 14 March 2019
	Newcastle 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 **Tel: +44 (0)191 2418715** **E-Mail: Amritjit.Singh@nuth.nhs.uk** **Website: http://www.newcastle-mitochondria.com**
Testing performed at the above address only	

Laboratory location:

Location details		**Activity**	**Location Code**
Address	**Contact**		
NHS Highly Specialised Service for Rare Mitochondrial Disorders Wellcome Trust Centre for Mitochondrial Research 4th Floor Cookson Building The Medical School Newcastle University Framlington Place Newcastle upon Tyne NE2 4HH	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	Mitochondrial Disorders	Using In-house documented methods incorporating manufacturer's instruction where relevant.
	Enzyme histochemistry/histology	
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 Skin	Sectioning of frozen whole tissue for histological and enzyme histochemical analysis. Preparation of frozen tissue sections for histopathological and histochemical assessment of mitochondrial function	Sectioning frozen material using the cryostat ID 5843 Sample preparation for histochemistry ID 7163 Preparation of primary culture from skin biopsy ID 7098 Cell splitting – subculture technique ID 7092
	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 ID 6397 Cytochrome *c* oxidase ID 6394 Succinate dehydrogenase ID 6413 Sequential Cytochrome *c* oxidase-Succinate dehydrogenase ID 6395 Modified Gomori Trichrome stain ID 6396 And: Bright OTF 5000 Cryostat ID11014 Zeiss Axiovision Image Acquisition System ID 7351

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. (cont'd)
	<u>Respiratory Chain enzyme measurements</u> Biochemical measurement of mitochondrial respiratory chain enzyme activities for (including combinations):	
Mitochondrial fractions from skeletal or cardiac muscle and fibroblasts	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 ID 7164 Preparation of Mitochondrial Fractions – skeletal or cardiac muscle ID 7165 Preparation of Mitochondrial Fractions – pig heart muscle ID 7167 Biochemical assay of Citrate Synthase ID 7971 Biochemical assay of respiratory chain complex I activity ID 7657 Biochemical assay of respiratory chain complex II activity ID 7758 Biochemical assay of respiratory chain complex III ID 7957 Biochemical assay of respiratory chain IV complex ID 8878 Preparation of Medium A and Medium B ID 7168 Preparation of mitochondrial fractions – liver ID 7166 Preparation of stock buffers for biochemistry assays ID 7369

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
Mitochondrial fractions from skeletal or cardiac muscle and fibroblasts (cont'd)		And: Sorvall Lynx 4000 centrifuge ID 20427 Cary Spectrophotometers and circulators ID 20426dividing with citrate synthase activity Using In house procedures: Preparation of Mitochondrial Fractions – fibroblasts and myoblasts ID 7164 Preparation of Mitochondrial Fractions – skeletal or cardiac muscle ID 7165 Preparation of Mitochondrial Fractions – pig heart muscle ID 7167 Biochemical assay of Citrate Synthase ID 7971 Biochemical assay of respiratory chain complex I activity ID 7657 Biochemical assay of respiratory chain complex II activity ID 7758 Biochemical assay of respiratory chain complex III ID 7957 Biochemical assay of respiratory chain IV complex ID 8878 Preparation of Medium A and Medium B ID 7168 Preparation of mitochondrial fractions – liver ID 7166 Preparation of stock buffers for biochemistry assays ID 7369 And: Sorvall Lynx 4000 centrifuge ID 20427 Cary Spectrophotometers and circulators ID 20426

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. (cont'd)
Human tissue – skeletal and cardiac muscle	<u>Quadruple Immunofluorescence</u>	
	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 ID 18103 Quadruple analysis online software tool ID 18104 Quadruple immuno analysis ID 18105 Quadruple Immunofluorescence respiratory chain complexes ID 18106 And: Zeiss Axiovision Image Acquisition System ID 7351 Mat Lab (computer analysing system, MathWorks®) Bright OTF5000 Cryostat Jenway pH Meter Obaus Adventurer Balance

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. (cont'd)
	<u>Molecular Genetics</u>	
Whole blood (including cord blood) Buccal scrapes Fibroblasts Myoblasts Urine Solid tissue (including, but not limited to, muscle, heart and liver) Hair FFPE tissue (paraffin blocks) Single cell blastomeres Extracted DNA samples	Analysis of mitochondrial DNA and nuclear DNA (mtDNA and nDNA, respectively) for clinically significant sequence changes. Analysis of mtDNA for large-scale rearrangements and copy number variation. Analysis of cDNA (generated from RNA) for splicing variants.	Extraction of DNA and RNA Using in house procedures: Preparation of primary culture from skin biopsy ID 7098 Cell splitting – subculture technique ID 7092 Specimen reception and booking – in procedure ID 7162 Extraction of DNA from blood using EZ1 Advanced Workstation ID 6539 Extraction of DNA from buccal using EZ1 advanced workstation ID 6540 Extraction of DNA from cultured cells using EZ1 Advanced Workstation ID 6541 Extraction of DNA from tissue using EZ1 Advanced Workstation ID 6542 Extraction of DNA from urine pellet using EZ1 Advanced Workstation ID 6543 Extraction of RNA from patient fibroblasts ID 15944 EZ1 Advanced Workstation – MDS ID 7217 Good practice guidelines – DNA extraction ID 9755 Blastomere lysis and Pyrosequencing PCR ID 26700

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) Molecular Genetics (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant. (cont'd)
Whole blood (including cord blood) Buccal scrapes Fibroblasts Myoblasts Urine Solid tissue (including, but not limited to, muscle, heart and liver) Hair FFPE tissue (paraffin blocks) Single cell blastomeres Extracted DNA samples	Analysis of mitochondrial DNA and nuclear DNA (mtDNA and nDNA, respectively) for clinically significant sequence changes. Analysis of mtDNA for large-scale rearrangements and copy number variation. Analysis of cDNA (generated from RNA) for splicing variants. (cont'd)	Extraction of DNA and RNA (cont'd) Using in house procedures: Microdissection and lysis of single muscle fibres ID 10810 Nanodrop 1000 – measuring nucleic acids ID 7195 And: UV sterilizing PCR cabinet Zeiss PALM laser microdissection microscope ID 10810 Qiagen EZ1 Advanced Workstation ID 7217 NanoDrop ND-1000 Spectrophotometer ID 7195
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection of known or unknown sequence changes	Fluorescent Sanger sequencing and capillary electrophoresis (mtDNA analysis using SeqScape software; Applied Biosystems) (nDNA analysis using Mutation Surveyor software; SoftGenetics), Analysis of sequence variants using Alamut software (Interactive Biosoftware). Using in house procedures: Cycle sequencing and capillary electrophoresis using ABC3130xl ID 23675 Use of Mutation Surveyor (version 4.09) ID 12260 Sequencing analysis using SeqScape 3 (version 3.0) ID 12261 Nomenclature for the description of sequence variants ID 12355 Investigation of sequence variants ID 12277 mtDNA primers ID 12271

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. (cont'd)
	Molecular Genetics (cont'd)	
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source (cont'd)	Detection of known or unknown sequence changes (cont'd)	Fluorescent Sanger sequencing and capillary electrophoresis (mtDNA analysis using SeqScape software; Applied Biosystems) (nDNA analysis using Mutation Surveyor software; SoftGenetics), Analysis of sequence variants using Alamut software (Interactive Biosoftware). (cont'd) Preparation of reagents for molecular biology – stock chemicals ID 12280 Primer design and ordering SOP ID 12275 Primer log ID 12276 MAN0006735 Ion AmpliSeq DNA and RNA Library Preparation_Revision D.0 ID 20526 MAN0006775 Ion AmpliSeq DNA Library Preparation with 1 or 2 pools qPCR Quant Quick Ref_Rev B.0 ID 20527 MAN0006943 Ion AmpliSeq DNA Library Preparation Qubit2 or Agilent2100 Quant Quick Ref_Rev B.0 ID 20528

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. (cont'd)
	Molecular Genetics (cont'd)	
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection of known or unknown sequence changes (cont'd)	Next generation sequencing using the Ion Torrent PGM platform (analysis using Torrent Suite; Life Technologies). Analysis of sequence variants using Alamut software (Interactive Biosoftware). MAN0007044 Prepare Amplicon Libraries Req Frag Using the Ion Xpress Plus Fragment Library KitID 20529 MAN0015802 IonLibrary Taqman Quantitation Kit ID 20554 MAN0013432 Ion AmpliSeq Library Preparation on the Ion Chef System ID 20530 MAN0013433 Ion AmpliSeq Library Preparation on the Ion Chef System ID 20531 MAN0014571 Ion PGM HiQ View Chef KitsID 20532 MAN0014572 Ion PGM HiQ View Chef Kits ID 20533 MAN0014579 Ion PGM HiQ View OT2 Kit ID 20534 MAN0014580 Ion PGM HiQ View OT2 ID 20535 Ion Torrent One Touch 2 ID 20534 / ID 20535 Ion Torrent PGM instrument ID 20536 / ID 20537 Ion Chef instrument ID 20538 / 20530 / 20531 / 20532 / 20533 Next generation sequencing data analysis ID 21571 And: PCR blocks ID 12264, 14341, 8364, 8455

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. (cont'd)
	Molecular Genetics (cont'd)	
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection of known or unknown sequence (cont'd)	Pyrosequencing (analysis using Pyromark Q24 software; Qiagen). Analysis of sequence variants using Alamut software (Interactive Biosoftware). Manual preparation of mitochondrial DNA (mtDNA) amplicon libraries ID 20553 BioRad ChemiDoc MP imaging system ID 14343 Pyrosequencing assay design protocol ID 9977 Pyromark Q24 pyrosequencer ID 20418 Pyrosequencing – Principles and Process ID 23701 Blastomere lysis and Pyrosequencing PCR ID26700 Reporting results in Mitochondrial Diagnostic Service ID 10281 Checking of molecular genetic results ID 12342

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. (cont'd)
	Molecular Genetics (cont'd)	
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection of large-scale mtDNA rearrangements	Using three separate long-range PCR assays followed by agarose gel electrophoresis Using in house procedures: Long Range PCR of mtDNA ID 12305 Agarose gel electrophoresis ID 8796 Reporting results in Mitochondrial Diagnostic Service ID 10281 And: PCR blocks ID 12264, 14341, 8364, 8455 BioRad ChemiDoc MP imaging system ID 14343
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection of mtDNA copy number abnormalities	Using a Taqman® real-time PCR assay Using in house procedures: TaqMan real-time PCR for mitochondrial DNA depletion assay ID 9278 And: UV sterilising PCR cabinet Applied Biosystems StepOne Plus real-time PCR system ID 10236

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) Molecular Genetics (cont'd)	Using In-house documented methods incorporating manufacturer's instruction where relevant (cont'd)
DNA or RNA, either extracted from the sample types listed above in-house or received extracted from an external source	Detection and quantification of large-scale mtDNA rearrangements	Using a Taqman® real-time PCR assay in individual muscle fibres or single cells Using in house procedures: Real-time PCR for mitochondrial DNA deletions in single muscle fibres ID 10242 Microdissection and lysis of single muscle fibres ID10810 and 6395 And: UV sterilising PCR cabinet Applied Biosystems StepOne Plus real-time PCR system ID 10236 Zeiss Axioplan Microscope ID 7351 Nikon TS100 Microscope ID 22133
cDNA, generated from RNA extracted from the sample types listed above in-house or received extracted from an external source	Presence, absence or size of specific fragment of cDNA	Reverse transcription PCR (RT-PCR). PCR sizing analysis and fluorescent Sanger sequencing RNA analysis of potential splicing variants ID 15946 Reporting results in Mitochondrial Diagnostic Service ID 10281 Nomenclature for the description of sequence variants ID 12355 Cycle sequencing and capillary electrophoresis using ABC3130xl ID 23675 Reporting results in Mitochondrial Diagnostic Service ID 10281 And: PCR blocks ID 12264, 14341, 8364, 8455 BioRad ChemiDoc MP imaging system ID 14343 ABI3130 capillary electrophoresis instrument ID 9283

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