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Service information: Rett Syndrome (RTT)

Gene/Locus: *MECP2* (Xq28) **OMIM:** #312750; *300005 (*MECP2* gene)

Referrals: Clinical Geneticists, Paediatricians, Neurologists, Other Relevant Specialties

Testing: Diagnostic testing offered in clinically affected patients (Rett syndrome or *MECP2* duplication syndrome). Carrier testing in females with a family history of pathogenic or likely pathogenic *MECP2* mutation if referred by Clinical Genetics

Target Reporting Times:

• Diagnostic	56 calendar days	28 calendar days for MLPA only
• Carrier testing	28 calendar days	
• Urgent testing*	3 calendar days	Prenatal diagnosis/carrier testing in pregnant women

***Please contact the laboratory if urgent testing is required**

Test Details:

- **Diagnostic screening of *MECP2*:** Next generation sequencing (NGS) of all coding sequence and intron/exon boundaries of *MECP2*. Confirmations and repeats tested using Sanger sequencing.
- **Testing for known pathogenic and likely pathogenic variants** in individuals with a family history of *MECP2* mutations, by Sanger sequencing or MLPA, as appropriate.

Service Details:

Sequencing should detect 85-90% of pathogenic and likely pathogenic variants found in classic Rett syndrome (RTT) and 30-40% of pathogenic and likely pathogenic variants in atypical RTT (Fukuda et al Brain & Development 27 (2005) 211-217). Large *MECP2* deletions can account for approximately 8% of classic RTT cases. However, deletions and duplications only account for 3% of mutations in atypical RTT (*MECP2* Related disorders Gene Reviews). Screening of other genes or gene panels can also be considered in patients with atypical RTT. This testing is available at another laboratory; however, the funding for this testing will need to be provided by your department. It is estimated that the prevalence of *MECP2* duplication syndrome is ~1% in males with moderate to severe learning disabilities (*MECP2* Duplication Syndrome – Gene Reviews).

Sample Requirements:

- **EDTA** blood sample (≥4 ml), labelled with patient's **full name**, **date of birth** and **NHS number**, or genomic DNA (≥7.5 µg at ≥50 ng/µl). Please send two separate blood/DNA samples for presymptomatic testing if possible.
- Samples should be accompanied by a **fully** completed referral card which should include the patient's full name, date of birth and NHS number.
- Please also include details of the gene(s) to be tested, relevant clinical details and full details of the referring clinician and centre.
- Familial positive control samples are required for presymptomatic testing, if available.

Consent:

Please note that in submitting a sample, it is the responsibility of the clinician to ensure that consent has been taken i) for testing, ii) for storage, and iii) for the use of this sample and the information generated to be shared with the patient's relatives and their health professionals. Following testing, a sample of the patient's DNA may also be used anonymously to validate new tests and for internal quality control purposes.

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MLPA testing:

Dosage analysis only by MLPA can be offered for MECP2 (P015).

Next generation sequencing (NGS) testing information.

NGS of all coding sequence and intron/exon boundaries (-30 to +15) of selected genes using a Sophia Genetics Custom HCS Panel and Illumina MiSeq platform. Data analysis using MiSeq Reporter software for FASTQ file generation followed by Sophia DDM for alignment of reads to genome build GRCh37, coverage analysis and variant calling.

The target region is covered at a minimum depth of 30x. Thresholds for variant calling: ≥15% total reads for indels and ≥20% total reads for single nucleotide variants (SNVs). Based on in-house test validation the analytical sensitivity of this testing is estimated to be greater than 98.6%. Variant confirmations and repeats are performed using Sanger sequencing. Copy number variation (CNV) confirmation by multiplex ligation-dependent probe amplification (MLPA).

If information on the coverage of individual genes is required, please contact the laboratory. Variants will be classified based on the ACMG variant interpretation guidelines (Richards et al (2015) Genet Med. 17:405-24; Ellard et al (2017) ACGS Best Practice Guidelines for Variant Classification 2017).Sequence variants defined by the laboratory as benign or likely benign variants are recorded but not reported. In some cases variants of uncertain clinical significance with no convincing evidence for pathogenicity may not be reported. NGS sequence data on other genes on the Sophia Genetics Custom HCS Panel that are not requested at the time of referral will be stored but not analysed.

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