The European Molecular Genetics Quality Network

EMQN Pilot External Quality Assessment Scheme for Silver-Russell Syndrome (SRS) (2015)

SCHEME REPORT

Authorisation/Approval
EMQN is a UKAS accredited provider of EQA services – this scheme is NOT covered under the scope of its accreditation. This document has been authorised / approved on behalf of EMQN by:

Simon Patton (EMQN Director) on 02 June 2015

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02 June 2015

Dear Colleague,

Thank you for participating in the EMQN pilot EQA scheme for **Silver-Russell Syndrome** testing. This document is the final report summarising the results of the scheme- your Individual Laboratory Report (ILR) is available from your website account. The key findings raised by the scheme this year are shown in the table below. For more detailed information about the scheme, please see the remainder of the document.

KEY FINDINGS FROM SCHEME

CATEGORY	COMMENTS
Genotyping	1. **Case 1:** Two laboratories missed the mutation (6.4%) and seven (22.6%) only got it partly right. Three out of 31 laboratories had problems with the DNA and were therefore not marked. 2. The vast majority (n=27) of laboratories used only MS-MLPA (87.1%); two laboratories used second methods to confirm their MS-MLPA results, one laboratory used MS-MLPA and MS-PCR, one laboratory MS-MLPA and MS-SNuPE; two laboratories used only bisulphite treatment and subsequent MS-PCR followed by high-melting curve analysis. 3. Please keep in mind that it is generally recommended in molecular genetic diagnostics to confirm a positive result by an independent second method whenever possible. 4. **Case 2:** Three laboratories missed the mutation (9.7%) and one got it partly right (3.1 %). Four out of 31 laboratories had problems with the DNA and were therefore not marked. Methods used were the same as in case 1. 5. **Case 3:** The majority of laboratories got the right result with the method used. Two laboratories (6.5%) had a critical genotyping error and one laboratory got the result partly wrong. Again, two laboratories had problems with the DNA and were therefore not marked. 6. Unfortunately, only 8 out of 31 laboratories (25.8%) performed upd(7)mat testing. As 7-10% of all SRS cases are upd(7)mat, we think this figure is too low. Laboratories did not get a reduction for not performing upd(7)mat testing in this scheme but we strongly recommend including this important test in standard SRS testing. Methods used were the same as in case 1.
Interpretation	7. **This was a pilot EQA scheme and therefore interpretation was assessed, but not assigned a mark.** 8. **Case 1:** Three laboratories did not ask for parental blood samples to determine (i) whether the duplication is *de novo* and (ii) its extent. Eight laboratories did not point out that there is a recurrence risk to further offspring. A few interpretive mistakes (e.g. no genetic counselling offered; not said that result confirms SRS) were made by single laboratories. 9. **Case 2:** A few laboratories got reductions because they did not offer genetic counselling to the family. Even though this is not a high-risk situation, we feel that the underlying complex genetic mechanism should be explained to the family in the setting of genetic counselling. The family will also have to understand that this genetic variation does not necessarily lead to an increased recurrence risk. Seven laboratories did not point out that the recurrence risk in this case is low. In this year`s pilot scheme we did not deduct marks in case the risk of Multilocus Imprinting Disturbance (MLID) was not mentioned but we want to emphasize that an increasing number of patients with this defect has recently been found and that it should indeed be discussed. Please make sure that you are up-to-date in this respect. 10. **Case 3:** Among the laboratories that did not test for upd(7), a single one did not recommend this test as the next diagnostic step and therefore this lab got a reduction. However, as indicated above, we feel that a lab offering SRS testing should also offer upd(7)mat testing. Four laboratories did not say that upd(7)mat has a low recurrence risk except in the case of a parental chromosomal rearrangement for which testing should be recommended. Two laboratories did not indicate which assay they used for the diagnostics
Nomenclature	11. It is EMQN policy to use HGVS mutation nomenclature – this includes use of RefSeq's.
Reporting	12. In general, we only had very few deductions in this field. However, we would like to point out that many laboratories did not state the patients´ gender as it should be done and that in many cases page numbering was either missing or inadequate. The patient´s name and page numbers should be given on every page of a report (e.g. page 1 / 2 of report….). This is according to ISO 15 189. Reports should be restricted to one page whenever possible – and it usually is possible! 13. Be consistent in the naming of loci, do not use IC1/ IC2 and H19/ KCNQ1OT1 independently in one report without saying which gene is located in which imprinting centre. 14. Try to be concise in your reports; highlight the important message; write reports that clinicians will also

	understand, i. e. give clear information and omit redundancies. When giving references from the literature, make sure that they are up-to-date and precise. 15. For case (3), one laboratory wrote two separate reports, one saying that no chromosome 11 aberration was found and one reporting upd(7)mat. This was rather unusual and we recommend giving all-important information in a single report so that no information will be lost.

Clerical Accuracy and Patient Identifiers	16. No major points of concern were identified.

STRUCTURE OF THE SCHEME

This pilot external quality assessment scheme was designed to assess the ability of participating laboratories to correctly genotype cases suspected of having SRS, the ability to correctly interpret the genotypes and initiate, if necessary, further analysis. The DNA samples distributed were prepared from cell lines established from peripheral blood samples and their genotypes independently validated on the same batch of DNA in two separate laboratories. Diagnostic requests for the three (mock) clinical cases were sent together with the samples.

CASES AND EXPECTED GENOTYPES

CASE	PATIENT NAME	DATE OF BIRTH (dob)	REASON FOR REFERRAL	CONFIRMED RESULT
1	Luka TABOR	02/09/2014	Luka TABOR is referred from neonatal intensive care following caesarean section due to severe IUGR. His birth length and weight are -3SD for his gestational age, his head circumference -1.5SD, and his facial features consistent with Silver-Russell syndrome.	ICR1 hypomethylation ICR2 hypermethylation (duplication of maternal allele*)
2	Emilia MIRABELLO	26/04/2012	Emilia MIRABELLO has a clinical diagnosis of Silver-Russell syndrome, with failure to thrive, relative macrocephaly, triangular facies, mild limb asymmetry and feeding difficulties.	ICR1 hypomethylation
3	Thomas SELIGMAN	14/02/2009	Thomas SELIGMAN was born at 28 weeks of gestation with mild hypospadias. He now has symmetrical short stature and extreme failure to thrive, and is dependent on enteral feeding. Please test for SRS.	UPD7mat

NB: there is currently no standardised nomenclature for the loci affected by methylation disturbance in SRS, nor for the nature and degree of methylation disturbance. Until such standardisation exists, a variety of synonyms remain acceptable, such as: ICR1 = H19; ICR2 = LIT1 = KCNQ1OT1 = KvDMR; hypermethylation = gain of methylation; hypomethylation = loss of methylation; UPD7 = mat UPD7 = UPD7mat

ASSESSMENT

The team involved in helping to organise and assess the scheme results were as follows:

COLLEAGUE	COUNTY	ROLE
Katja Eggermann	Germany	Scheme Organiser
Karen Gronskov	Denmark	Assessor
Deborah Mackay	United Kingdom	Assessor
Irene Netchine	France	Assessor

The assessment consisted of three categories: genotyping accuracy, correct biological and clinical interpretation of results and patient identifiers/clerical accuracy. For assessment, in addition to the genotype, we expected a biological interpretation of the observed nucleotide change and we further review the clinical interpretation given. Clinical interpretation refers to the immediate consequence of the observed mutation for the patient and her family. The reporting format and style were reviewed and not marked. The full score for genotyping and patient identifiers/clerical accuracy categories was 2.00 marks. **This was a pilot EQA scheme and therefore interpretation was assessed, but not assigned a mark.**

CASE	CATEGORY	CRITERIA	MARKS
1	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing comment - this result confirms a clinical diagnosis of SRS/RSS	Comment only
		Missing comment - the molecular cause of SRS/RSS is a duplication of the SRS locus (or H19, or ICR1) on the maternal allele	

		Missing comment - request for parental samples to determine whether the duplication is de novo, and its extent	
		Missing comment (in the case of methods other than MS-MLPA) - the methylation pattern observed is consistent with a duplication on the maternally-derived allele or UPD11mat	
		Missing comment (In the case of methods other than MS-MLPA) - copy number analysis in the proband and parents (mother) is recommended to confirm presence of a duplication and whether it is inherited or de novo	
		Missing comment - there is a risk of recurrence in other offspring. Prenatal testing may be offered	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

2	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing comment - this result confirms a clinical diagnosis of SRS/RSS	Comment only
		Missing comment the molecular cause of SRS/RSS is hypomethylation of ICR1	
		Missing comment - risk of recurrence is low	
		Comments: risk of multi-locus imprinting disturbance may be mentioned and further testing suggested	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

3	**Genotyping**	**Correct genotype**	2.00
		Deductions: Critical genotyping error	-2.00
		Genotype mis-positioned or mis-called (e.g. incorrect base/amino acid detected)	-0.50
		Error in HGVS nomenclature which could be mis-interpreted	-0.50
		Not correctly using HGVS nomenclature (for either nucelotide or protein)	-0.50
		RefSeq missing / incorrect / inconsistent	-0.50
		RefSeq version number missing / incorrect / inconsistent	-0.25
	Biological and clinical Interpretations	Missing comment (if UPD7 testing is not performed) - recommend UPD7 testing	Comment only
		Missing comment (if UPD7 testing is not performed) - UPD7 accounts for 5-10% of SRS	
		Missing comment - UPD7 has a low recurrence risk except in the case of genetic rearrangement in parents; offer genetic counselling to parents	
	Patient identifiers and clerical accuracy	See standard deductions below.	2.00

STANDARD DEDUCTIONS		
	Standard deductions for patient identifiers and clerical accuracy	*Points were deducted for:* • Incorrect DOB (any error) (-1.00) • Spelling errors in patient name (-0.50) • Incorrect or missing patient gender (-0.50) *Minor points (not leading to deduction of mark)* • Date of referral / arrival noted • Missing - title of the report • Missing - identity of the laboratory performing the analysis and issuing the report • Missing - full date of the report • Missing - page numbers indicating the total number of pages (essential when multiple pages are used) • Missing - name and address of the physician referring the patient • Signature of the report by two authorised persons • Laboratory reference • Reason for referral restated • Clear and concise report

PARTICIPATION

This year, 31 laboratories from 14 countries registered for the scheme; all returned reports. The participating countries are shown in the Appendix (figure 1).

RESULTS

Overall quality was satisfactory and we had the pleasure to see some excellent reports. Most often, the genotypes were correctly found, reported and interpreted in their clinical context. No marks were deducted for interpretation.

METHODOLOGY

The majority of laboratories used methylation sensitive MLPA, sometimes in combination with another method (see Figure 2). Most laboratories got the materials to work however there were a number of additional requests for material. We did extensive validation on the materials and they performed to specification however we suspect that the additional requests were because the methylation patterns sometimes appear slightly unusual due to them being derived from cell lines, rather than a patient blood sample. However, only one laboratory did not submit any reports due to difficulties with genotyping. In total, three laboratories did not submit reports for case 1, four for case 2 and two for case 3. Therefore we conclude that no sample presented insuperable difficulties to all laboratories. However, DNA preparation will be re-addressed in the coming year and the pilot repeated, to attempt to secure higher success rates with the samples.

APPEALS PROCEDURE

Performance criteria do not apply and there is no appeals procedure against the marking as this is a pilot EQA scheme. Please remember that the primary aim of this EQA is to be educational, not punitive, and that we are trying to assist laboratories in their continuous efforts towards a higher quality of service.

CONFIDENTIALITY

The fact that your laboratory participates in EMQN schemes is not confidential. However, the raw data and performance scores are. Your laboratory information is confidential between you and the EMQN office (and in exceptional circumstances the Scheme Organiser and Management Board). Only your laboratory's allocated unique EMQN reference number will identify its scores if published within this summary report.

FINAL COMMENTS

Finally, the assessors wish to cordially thank the participants for their hard work, prompt returns and their co-operation during this exercise. We have seen a quite high technical standard of mutation analysis and we have had the pleasure to review some excellent and many good reports. We hope that labs will take on board any comments made by the assessors to help improve the scores in future schemes. Regular participation is associated with improved interpretation performances. We therefore encourage all labs to participate every year and we look forward to your participation in the 2016 scheme that will again be announced by the EMQN office in Manchester. Registration will be through the EMQN web site as before.

With our best wishes,
Yours

Katja Eggermann, Karen Gronskov, Deborah Mackay and Irene Netchine

APPENDIX

Figure 1: *Scheme participation*

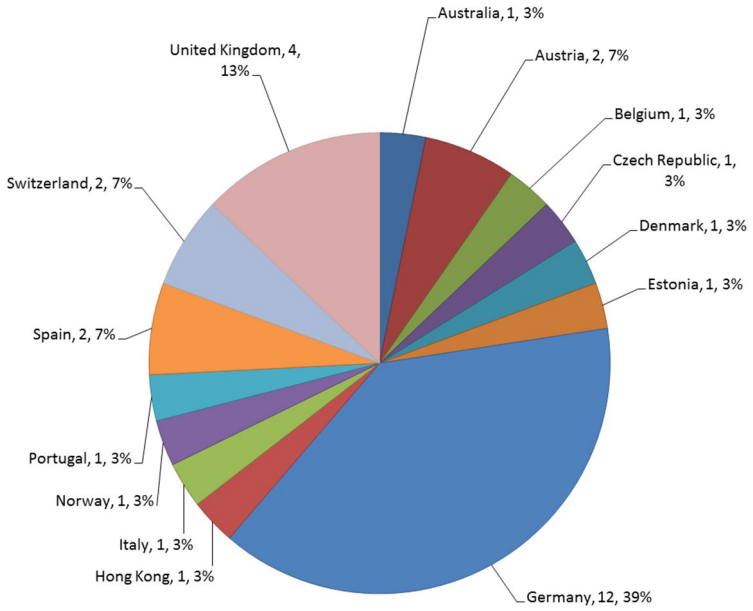
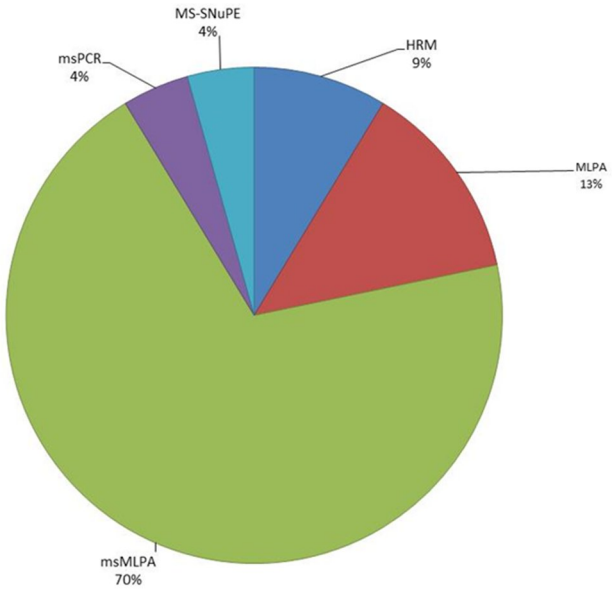


Figure 2: *Methods used* [1]



[1] This figure represents a summary of all the different methods used in the scheme. It DOES NOT depict the combination of methods used by different labs in their testing approach for this disease indication.

Table 1: *Mean genotyping, interpretation and patient identifiers / clerical accuracy results*

	AVERAGE SCORES PER CASE		
	Genotyping	**Interpretation**	**Patient identifiers / Clerical accuracy**
Case 1	1.73	Not marked	1.98
Case 2	1.76	Not marked	1.95
Case 3	1.84	Not marked	1.97
Mean	**1.78**	**Not marked**	**1.97**

Table 2: *Genotyping error rates*

	GENOTYPING ERRORS PER CASE		
	No. of cases completed	**No. of errors**	**Error rate (%)**
Case 1	31	2	6.4
Case 2	31	3	9.6
Case 3	31	2	6.4
Total	**93**	**7**	**7.5**

References:

- *None*