Lynch syndrome; hereditary non-polyposis colon cancer, HNPCC (LYNCH) EQA 2025

Post-appeals summary scheme report

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28 August 2025

Dear Colleague,

This external quality assessment (EQA), Lynch syndrome (hereditary non-polyposis colon cancer, HNPCC) (LYNCH) is run by EMQN CIC. The EQA assessment included the scoring of genotype, interpretation and clerical accuracy. This EQA summary scheme report includes assessment data using harmonised marking criteria. EMQN CIC is responsible for this EQA, and all correspondence related to it should be directed to us.

The assessment is now complete and your individual laboratory scores have been agreed by the assessors. Please go to your EMQN CIC website account to download your Individual Laboratory Report (ILR):

- EMQN CIC (www.emqn.org): select the 2025 "LYNCH" EQA.

A certificate of participation will be available after the appeals process closes and final results are published, along with the final ILR and scheme summary report.

EQA design and purpose

The aim of this EQA is to assess the testing accuracy (genotyping), and reporting (biological and clinical interpretation of the test result and overall report content and clerical accuracy) for Lynch syndrome and to help make improvements using a combination of assessment and educational feedback (expert commentary) via both Individual Laboratory Reports (ILRs) and this EQA Scheme Summary Report when required.

The EQA design meets these objectives by assessing the ability of the participating laboratories to:

- Correctly genotype variants in Lynch syndrome associated genes,
- Interpret the results in response to the clinical referral in a clear and concise format,
- Correctly use internationally accepted standard nomenclature, and
- Provide appropriate and accurate patient and sample identifiers.

This summary scheme report contains information from the cohort of participants including geographical spread, methodologies employed, common errors, learning points and scheme statistics to allow participants to benchmark their results.

Summary report on behalf of the assessment team

Continued Performance

- There were no laboratories that received poor performance for this year's EQA, which is an improvement upon last year where three laboratories (3/159, 1.99 %) received poor performance.
- The overall performance for genotyping was very high with an average of 1.99 across all cases. This is a slight improvement on 2024, where the average score was 1.97.
- The overall performance for interpretation remains good with an average of 1.92 in 2025. This is a slight improvement on 2024, where the average score was 1.89.
- The overall performance of patient identifiers and clerical accuracy remained very high with an average score of 1.95 in 2025 which is a slight decrease compared to 2024.

All Cases

Genotyping

- Full marks in all cases and all categories were achieved by 52 (34.7 %) laboratories.
- No result was evaluated as a critical genotyping error.
- Full marks for genotyping in all three cases were obtained by 124 (82.7 %) laboratories; 419/445 (94.2 %) of marked genotypes were fully correct, without any deduction. The main reason for deduction in genotyping was a minor HGVS error in case 1.

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- Three laboratories failed to indicate zygosity in all three cases. Please keep in mind, that information about zygosity is an important part of genotyping and cannot be substituted by variant allele frequency (VAF) detected by NGS.
- EMQN CIC recognizes that the MANE initiative[1] is still in development and will not penalize participating laboratories for using the correct LRG reference in the 2025 scheme rounds. However, from 2026 onwards, laboratories can expect to receive a marking deduction for using ONLY the LRG reference to report variant descriptions.

Interpretation

- No critical interpretation errors were observed. Biological and clinical interpretation were excellent in cases 1 and 2, where pathogenic (ACMG class 5) variants were detected. Similarly to previous years, reporting of a negative result (absence of the variant) appeared to be the most difficult. Recurrent minor errors and omissions led to frequent deductions. Again, some laboratories did not provide clinical interpretation in all three cases, although they did not declare participation in "genotyping only" for the EQA scheme.
- Reasons for deduction in individual cases are shown below.

Clerical Accuracy

- Full marks for clerical accuracy in all three cases were obtained by 123 (82 %) laboratories. Deductions were frequently systematic, e.g. appearing in all three reports submitted by the laboratory. Nine laboratories failed to provide sample batch number (-0.5 points), ten laboratories failed to provide patient's identifiers on each page of the report (-0.2 points), six laboratories did not report sample batch number (-0.5 points), and eight laboratories failed to provide the date of sample receipt in all three reports. Thirty-one laboratories did not describe sample type correctly, mentioned DNA instead of a primary sample, e.g., a lymphoblastoid cell line (no point deduction).

Case 1

Genotyping

- This sample had a heterozygous *MSH6* variant: NM_000179.3:c.1135_1139del p.(Arg379Ter).
- There were no critical genotyping errors for case 1 this year. Full marks for genotyping were reached by 132 (132/150, 88 %) laboratories. Fifteen laboratories described the detected variant as p.(Arg379_Asp380delinsTer) instead of p.(Arg379Ter) (Minor HGVS error, -0.2 points).

Interpretation

- Full marks for interpretation were reached by 134 (134/149, 89.9 %) laboratories. The most serious reasons for deduction were: no clinical interpretation (-1.5 points), failure to mention genetic counselling (-0.5 points) and failure to suggest monitoring programme for the patient (-0.5 points).

Clerical Accuracy

- Full marks for clerical accuracy were reached by 128 (128/150, 85.3 %) laboratories.

Case 2

Genotyping

- There was no critical genotyping error in case 2 this year and full marks for genotyping were reached by 145 (145/150, 96 %) laboratories.
- This sample was heterozygous for *MLH1* variant: NM_000249.4:c.18_34del p.(Val7ArgfsTer18).

Interpretation

- Full marks for interpretation were reached by 134 (134/149, 89.9 %) laboratories. Major deductions were for reasons similar to case 1.

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Clerical Accuracy

- Full marks for clerical accuracy were reached by 127/150 (84.7 %) laboratories.

Case 3

Genotyping

- There were no critical genotyping errors for case 3 this year and full marks for genotyping were reached by 143 (143/145, 98.6 %) laboratories. Genotyping in case 3 was not marked for three laboratories, who classified the *MSH2* variant NM_000251.3:c.277C>T p.(Leu93Phe) as a class 3 (ACMG) variant, declared that according to their national guidelines a class 3 variant cannot be used for a predictive testing, and did not perform the analysis.

Interpretation

- Interpretation in case 3 appeared to be the most difficult. Case 3 required targeted analysis of an *MSH2* variant NM_000251.3:c.277C>T p.(Leu93Phe). This variant was originally classified by InSiGHT as class 4, likely pathogenic (ACMG). Application of modified ACMG criteria according to CanVIG-UK guidelines (Garret *et al*., 2024)[2] can change classification of the variant to class 3, a variant of uncertain significance (VUS)(ACMG). CanVIG-UK guidelines are not being implemented in all participating countries. Some countries have specific national guidelines, if a variant is classified as a VUS. These are only two of the possible reasons for differences in reporting among laboratories. Laboratories can share this uncertainty in classification with the referring clinician, describe the discrepancy or uncertainty in the report and suggest further analysis, if relevant, and an appropriate follow up. Many laboratories issued a report with full information for the clinical geneticist. On the other hand, many laboratories did not mention classification of the variant in the report at all. Complexity and seriousness of the situation and need for further improvement of classification rules can be deduced from these results. Classification of the *MSH2* variant NM_000251.3:c.277C>T p.(Leu93Phe) by laboratories was as follows:
    - 22 participants classified the variant as a VUS, class 3 (22/144, 15.3 %)
    - 55 participants classified the variant as likely pathogenic, class 4 (55/144, 38.2 %)
    - 27 participants classified the variant as pathogenic, class 5 (27/144, 18.8 %), and
    - 41 participants did not report the classification. Interpretation in case 3 was considered correct, if recommendations were consistent with classification of the variant.
- For "failure to provide adequate details of the assay, e.g., sensitivity and specificity of the method used" in a negative result, a 0.2 points deduction was given to 42 laboratories (42/144, 29.2 %).

Clerical Accuracy

- Full marks for clerical accuracy were reached by 127 (127/145, 87.6 %) laboratories.

Professional standards

Laboratories are assessed against the guidelines and relevant peer reviewed literature currently available references[3]. Other guidelines against which laboratory reports are assessed may include the international nomenclature HGVS[4] and ISO standards (ISO15189)[5].

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Assessment team

The assessment of participants' submissions was undertaken by a team of independent, expert assessors.

Table 1: Assessment Team

Assessors	Location	Role
Anna Krepelova	Czech Republic	Scheme Organiser
Katarina Baluchova	Germany	Assessor
Marco Graf	Germany	Assessor
Nils Rahner	Germany	Assessor
Stéphanie Baert-Desurmont	France	Assessor

Appeals

The Lynch 2025 Scheme Summary Report (pre-appeals)v1 was published on 17/06/2025. There were 27 appeals submitted against the marking of the scheme by 15 laboratories. These appeals were reviewed by the members of the scheme assessment team alongside the EMQN team. Twenty-three of these appeals were upheld, two appeals were partially upheld, and two appeals were rejected. The ILRs of every laboratory submitting an appeal were updated with the EMQN response and, where relevant, this report has also been amended.

We have changed a criterion in case 3, interpretation deduction of 0.25 for "No/insufficient evidence for classification of variant" to 0.0 "No statement for classification of familial variant e.g. "The familial pathogenic/likely pathogenic variant was not detected". The original purpose of the deduction was that the team would recommend including classification of the variant for predictive testing. The majority of laboratories (103/144, 71.5%) provided classification for the familial variant NM_000251.3:c.277C>T p.(Leu93Phe). Please refer to case 2 interpretation feedback for breakdown of classification.

If your laboratory has reported a critical error, you will receive a letter of poor performance. We request that you investigate the cause of this poor performance and report back to us within 3 months of the publication of this letter on actions taken to prevent any recurrence. Please complete our EQA Performance Investigation form which can be accessed from your EMQN website account by going to the "Schemes" tab and selecting the relevant EQA scheme(s).

Confidentiality

Details of our confidentiality policies can be found here: https://www.emqn.org/terms-conditions/ in section 4.6 Performance evaluation.

Subcontracted activities

Your EQA provider does not subcontract activities such as EQA planning, evaluation of performance or the authorization of reports. However, some activities are subcontracted, for example the preparation of materials may be performed by suitably accredited providers. Validation of EQA materials and technical advice for setting case scenarios and assessment of results is provided by the EQA team and expert centres.

If your laboratory has subcontracted part of the analytical process to another organisation / third party, this should be clearly stated on your clinical reports (ISO15189 REQ 6.8.2 and REQ 7.4.1.7)[3].

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Final comments

The assessment team would like to thank all participants for their hard work, prompt return of results and their co-operation during this exercise.

The purpose of the EQA service is to educate and facilitate the raising of standards. Assessors volunteer considerable time and effort to mark the submissions and to provide assistance to laboratories that may require improvement.

We look forward to your participation in the 2026 EQA scheme, and you will be notified by email when registration is available on the EMQN CIC website.

Thank you for participating in this EQA scheme and we hope you have found it a useful EQA exercise.

Kind regards,

Dr. Anna Krepelova

Scheme Organiser

On behalf of the Assessment Team

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APPENDICES

Rationale for clinical cases

Case 1

Ability to detect a variant in *MSH6* gene by NGS gene panel analysis, to describe it using correct HGVS nomenclature, biological and clinical interpretation,

Ability to issue a complete final report of a pathogenic variant in a diagnostic test.

Case 2

Ability to detect a variant in *MLH1* gene by NGS gene panel analysis, to describe it using correct HGVS nomenclature, biological and clinical interpretation,

Ability to issue a complete final report of a pathogenic variant in a diagnostic test.

Case 3

Ability to report absence of a familial variant in *MSH2* gene in a negative targeted/predictive test, with classification of the familial variant and clinical interpretation of the result.

Participation

Table 2: Participation data

Participation Details	Number
Number of registrations	151
Number of withdrawals	1
Number of laboratories that did not submit results	0
Total number of participating laboratories	150

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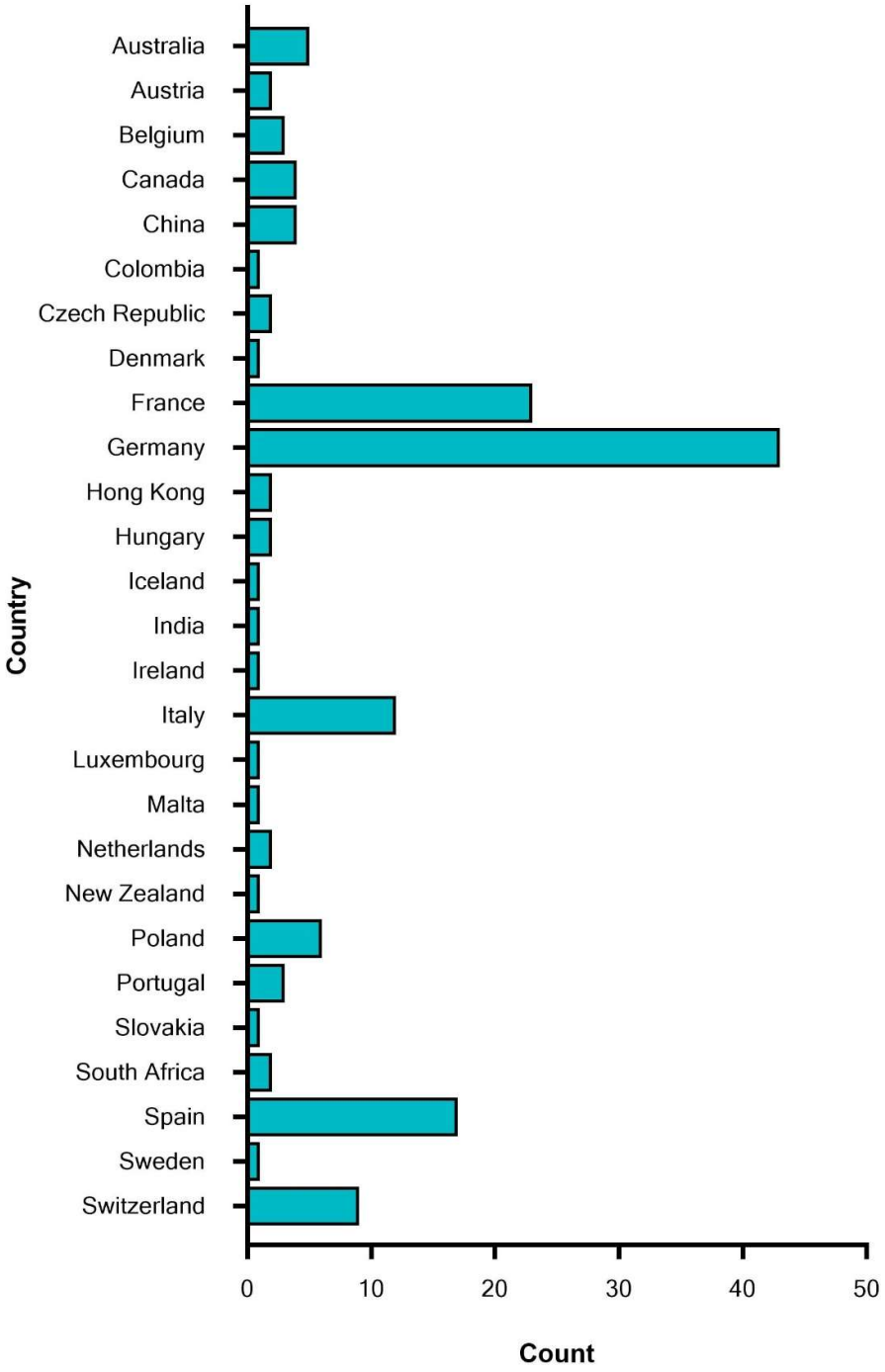
Figure 1: Participating countries



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Samples Provided and Validated Results

The participants received three DNA samples (in TE buffer) extracted from lymphoblastoid cell lines. The genotype of each EQA sample was validated independently using NGS and Sangers sequencing, in two different laboratories. Diagnostic requests for the three mock clinical cases were sent together with the samples (except for virtual cases, where samples were not provided). The expected results are shown in Table 3.

Table 3: EQA Sample details and validated results

Case	Name	Sex	Date of Birth (dob)	Referral Reasons	Validated Result
1	Ann TIGER	Female	01/03/1986	Ann Tiger has recently been diagnosed with synchronous endometrial and ovarian cancers. Endometrial tumour tissue showed MSI-high, loss of MSH6 protein expression and normal MLH1, PMS2, and MSH2 protein expression. Ann's maternal aunt died of endometrial cancer. The Consultant Clinical Geneticist has requested diagnostic NGS Lynch syndrome panel testing for Ann Tiger. Optional analysis if panel testing is not available in your laboratory: For the purpose of the EQA scheme, you may restrict analysis to the *MSH6* gene NM_000179.3:c.1050_1250. Please include analysis for flanking intronic sequence in accordance with your normal laboratory policy. If testing is restricted, you may assume all other required testing has already been performed and no other pathogenic/likely pathogenic variants including large duplications or deletions were identified.	Heterozygous for *MSH6* variant: NM_000179.3:c.1135_1139del p.(Arg379Ter)
2	John BARBER	Male	25/05/1984	John Barber has recently been diagnosed with right-sided colon cancer. Tumour tissue analysis revealed MSI, loss of the MLH1/PMS2 protein, *BRAF* V600E negativity, and no *MLH1* promoter hypermethylation. There is no family history of Lynch syndrome-associated tumours. The Consultant Clinical Geneticist has requested diagnostic NGS Lynch syndrome panel testing for John Barber. Optional analysis if panel testing is not available in your laboratory: For the purpose of the EQA scheme, you may restrict analysis to the *MLH1* gene NM_000249.4:c.-30_116.	Heterozygous for *MLH1* variant: NM_000249.4:c.18_34del p.(Val7ArgfsTer18)

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				Please include analysis for flanking intronic sequence in accordance with your normal laboratory policy. If testing is restricted, you may assume all other required testing has already been performed and no other pathogenic/likely pathogenic variants including large duplications or deletions were identified.	
3	Peter LOYD	Male	12/12/1999	Peter Loyd is a healthy male with a family history of colon cancer. His mother developed colon cancer at age 43 and his brother at age 27. NGS Lynch panel testing performed in your laboratory for Peter's brother revealed a germline variant NM_000251.3:c.277C>T p.(Leu93Phe) in the *MSH2* gene. The Consultant Clinical Geneticist has requested targeted predictive testing of this variant in Peter Loyd. For the purposes of this EQA you may assume that a second independently extracted DNA sample has been received and shows the same genotype as the one provided by EMQN.	NM_000251.3:c.277C>T p.(Leu93Phe): Variant in *MSH2* not detected

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Evaluation criteria of the reports

The assessment assigned marks to the genotyping accuracy and the interpretation of the results the laboratories provided in their reports. Patient details and clerical accuracy were also assessed. The full score for each category was 2.00. The assessors considered the accuracy, clarity and clinical relevance of the report issued to the referring clinician, with reference to available professional standards and publications.

Table 4: EQA Marking Criteria

Category	Category	Criterion	Deduction
All Cases	Genotyping	• Correct result reported	0
		• Critical genotyping error	2
		• Failure to indicate zygosity or incorrect zygosity	0.5
		• For Genotyping ONLY labs (clinical interpretation not provided): No indication of pathogenicity of variant detected / incorrect pathogenicity of variant detected	0.5
		• Including benign variants in a clinical report is not recommended	0
		• VUS not mentioned	0.5
		• Not using HGVS nomenclature	0.5
		• Major HGVS error (Genotype mis-positioned or mis-called e.g. incorrect base/amino acid detected)	0.5
		• Minor HGVS error	0.2
		• Use of legacy nomenclature not mentioned	0
		• Use of only legacy nomenclature	0.2
		• Non-standard transcript used	0.5
		• Transcript missing / incorrect / inconsistent	0.2
		• Transcript version number missing / incorrect / inconsistent	0
		• Error in use of HGVS brackets to show allele phase	0
		• Genome Build Not Provided	0.5
		• Not marked	0
		• Withdrawn from scheme	0
		• Test Failed	0
	Interpretation	• All essential interpretative elements provided	0
		• Critical interpretation error	2
		• No clinical interpretation of the genotype provided	1.5
		• Limited clinical interpretation	1
		• Misleading interpretive comment and/or generic interpretation which is misleading	1
		• Interpretation made in the wrong clinical context	0.5
		• No indication of pathogenicity of variant detected / incorrect pathogenicity of variant detected	0.5
		• Insufficient detail regarding variant classification system/evidence used to support classification	0
		• Clearly state the risk of an affected child applies to each pregnancy	0
		• Counselling and/or follow up is relevant but not mentioned in report	0.5
		• Failure to suggest monitoring programme or indicate patient is at risk of further disease	0.5
		• Failure to state which assay / methodology was used	0.5
		• Failure to provide adequate details of test performed (for example, limitations, LOD, accuracy, sensitivity and specificity) in relation to the suitability of the material provided	0.2
		• Failure to provide scope of the test(s) used i.e. which exons / codons / variants are covered	0.2
		• Clerical errors causing potential for patient harm e.g. incorrect/inconsistent use of the patient name	1

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		• Spelling and typographic error in the body of the text that changes the meaning of the report	1
		• Not marked	0
		• Not marked (due to critical genotyping error)	0
		• Withdrawn from scheme	0
		• Test Failed	0
	Clerical Accuracy	• All essential patient identifiers present and no significant clerical errors	0
		• Date of birth (dob) incorrect/missing	1
		• Patient name has small spelling error	0.5
		• Incorrect or missing patient sex	0.5
		• Failure to provide patient identifiers on each page of the report	0.2
		• No description of sample type or incorrect sample type	0
		• Reason for referral not restated	0
		• Errors in sample batch no. or no sample batch number provided	0.5
		• Failure to provide the dates of sample receipt / testing or reporting	0.2
		• Failure to anonymise report	0
		• Spelling and typographic error in the body of the text that does not change the meaning of the report	0
		• Very long report; a one page format is preferred to stick to the main points	0
		• Failure to provide a clear presentation of results	0
		• There is no evidence that the report was authorised i.e. report not signed by two people	0
		• Report should be stand-alone	0
		• Incorrect or No Pagination (e.g. Page X of Y)	0
		• Clear and concise report	0
		• Not marked	0
		• Not marked (due to critical genotyping error)	0
		• Withdrawn from scheme	0
		• Test Failed	0
Case 1	Interpretation	• No/insufficient evidence for classification of variant	0.25
Case 2	Interpretation	• The term “carrier” must not be used for AD disorder	0
		• No/insufficient evidence for classification of variant	0.25
Case 3	Interpretation	• No statement for classification of familial variant e.g. "The familial pathogenic/likely pathogenic variant was not detected"	0
		• The term “carrier” must not be used for AD disorder	0
		• Comment with deduction	0.25

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Results: Summary statistics

The mean scores for genotyping, interpretation, clerical accuracy and the total mean and median score for all participating laboratories are given below in Table 5. A summary of the number of critical errors per case is provided in Tables 6 & 7.

Non-participating laboratories were not marked nor included in this data.

Table 5: Mean Scores

Category		Case 1	Case 2	Case 3
Genotyping	Mean (SD)	1.97 (0.1)	1.98 (0.08)	2.0 (0.04)
	Median (SD)	2.0 (0.1)	2.0 (0.08)	2.0 (0.04)
Interpretation	Mean (SD)	1.94 (0.24)	1.95 (0.23)	1.87 (0.26)
	Median (SD)	2.0 (0.24)	2.0 (0.23)	2.0 (0.26)
Patient Identifiers & Clerical Accuracy	Mean (SD)	1.95 (0.13)	1.95 (0.13)	1.95 (0.14)
	Median (SD)	2.0 (0.13)	2.0 (0.13)	2.0 (0.14)

There were no critical genotyping errors or critical interpretation errors. Therefore, all laboratories achieved a satisfactory result.

Table 6: Critical Genotyping Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases completed	150	150	145*	445
Number of laboratories with full marks	132	145	143	420
Number of critical errors	0	0	0	0
Error rate (%)	0	0	0	0

*Three laboratories were assigned not marked for Case 3, one laboratory did not submit any results for all three cases and two laboratories did not submit reports for Case 3.

Table 7: Critical Interpretation Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases assessed	149	149	144*	442
Number of laboratories with full marks	134	136	92	362
Number of critical errors	0	0	0	0
Error rate (%)	0	0	0	0

*Four laboratories were assigned not marked for Case 3, one laboratory did not submit any results for all three cases and two laboratories did not submit report for Case 3.

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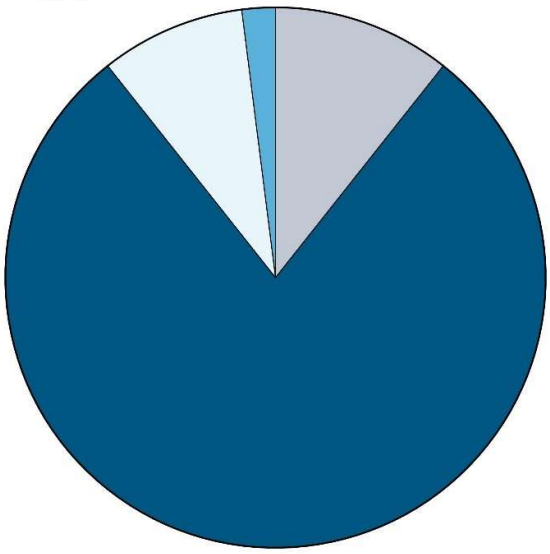
Results: Methodology used

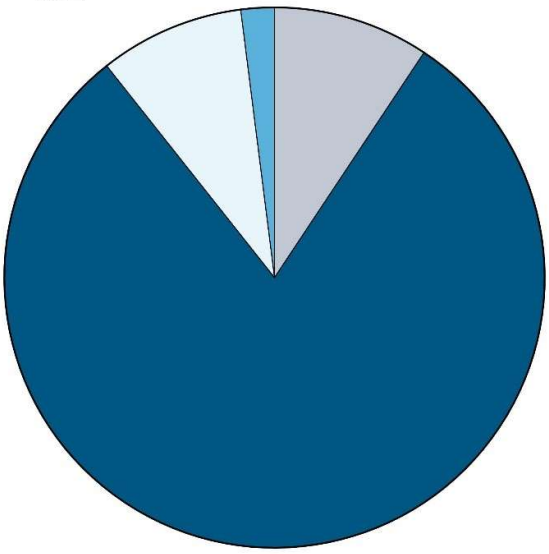
Case 1:



10.67% Sanger
78.67% NGS Targeted
8.67% NGS WES
2.00% NGS WGS

Total = 150

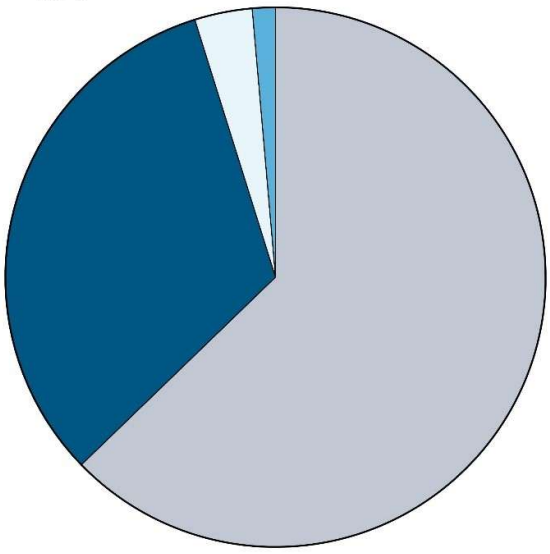
Case 2:



9.33% Sanger
80.00% NGS Targeted
8.67% NGS WES
2.00% NGS WGS

Total = 150

Case 3:



62.76% Sanger
32.41% NGS Targeted
3.45% NGS WES
1.38% NGS WGS

Total = 145

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Amendments to this Summary Scheme Report

Version	Page	Section	Change	Published
1	-	-	None	28/08/2025
2				
3				

Authorisation

This document has been authorised/approved on behalf of EMQN CIC by:

Dr. Simon Patton on 28th August 2025

CEO

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