Hereditary Breast / Ovarian cancer gene panel testing (HBOC panel) EQA 2024

Post-appeals summary scheme report

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22nd August 2024

Dear Colleague,

This external quality assessment (EQA), Hereditary Breast and Ovarian cancer panel testing (HBOC Panel) 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 2024 "HBOC Panel" EQA.

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 Hereditary Breast and Ovarian cancer panel testing 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 cases suspected of having hereditary breast or ovarian cancer using a panel test,
- 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 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

All Cases

Genotyping

- The average genotyping score was 1.99, a significant improvement on last year's average of 1.93. There was only one critical genotyping error across all reports (0.17% 1/586).
- A few reports did not indicate the zygosity of the variant which resulted in a deduction; variant allelic fraction is not sufficient in a germline context.
- Some of the panels used by laboratories participating in this scheme included genes that had little or no relevance to hereditary breast and ovarian cancer syndrome (or are still under investigation). Best practice guidelines for hereditary breast and ovarian cancer genetic testing have recently been published[1].
- EMQN supports the use of MANE Select and MANE Plus Clinical as denoted by the MANE initiative, for the standardization of variant annotation, interpretation and reporting[2]. Support for Locus Reference Genomic (LRG) reference sequences has been discontinued. While use of LRG reference sequences was still accepted this year, RefSeq or Ensembl transcripts specified by MANE are now preferred for sequence nomenclature. Laboratories have not been penalised for using LRG reference sequences this year, but are strongly encouraged to update to MANE transcripts for next year.

Interpretation

- The average interpretation score was 1.70, similar to last year's score of 1.72.

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- A general reminder that the term “carrier” of a variant should only be used for recessive disorders. Autosomal dominant diseases do not have carriers.
- Two of this year’s cases were male patients, some reports containing generic information on risk were acceptable although it is better to have specific recommendations for both females and males. It was incorrect to refer to ovarian cancer for this male patient (but acceptable to mention the risk in general). Also, it is critical to state when risk figures apply to females or males.

Clerical Accuracy

- A reminder that all reports should have an internal sample unique ID as well as an external reference ID when one is designated on the referral documentation (laboratory requisition).
- A large number of reports did not include a description of the sample type or source of DNA.
- Patient identifiers should be included on each page of the report.
- The font size used for methods and test limitations is frequently very small; with NGS panels it is understandable that inclusion of all the information required for the test methods and limitations often requires reports to exceed the ideal length of a single page.
- Consider report formatting: it is preferable to adapt report layouts to prioritize the sample information, results and interpretation first, followed by the methods, test limitations, references, etc.

Case 1

Genotyping

- Very well done to all participants – there were no critical genotyping errors for this case.
- The mean genotyping score for this case was 2.0. Only two laboratories received deduction, both were for HGVS errors.

Interpretation

- Many reports had *ATM* risk figures for females but failed to state this would not apply to the male patient. It is acceptable to include this information, but the gender to which the risks apply must be stated.
- This is considered a high-risk variant in a female, but the risks are unknown for a male. The *ATM* gene in general is a moderate risk gene but this specific variant is high-risk as ascertained for females.
- Risk to offspring for an autosomal recessive disorder should also be mentioned.

Clerical Accuracy

- No specific comment. See the “All Cases” section for general comments.

Case 2

Genotyping

- There were no critical genotyping errors for this case.
- A few participants reported the intronic splice variant in the wrong intron, i.e. stated the variant wasin intron 18 rather than intron 17, affecting exon 18.
- A general reminder that p.(?) is incorrect, p.? with no brackets is the correct nomenclature (brackets are not needed as the protein interpretation “?” is already an ‘unknown consequence’).

Interpretation

- Several laboratories reported the results for this male case in a female context. Ensure reports are specific for male patients.
- There was one critical interpretation error for this case, due to conflicting results in the report.

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Clerical Accuracy

- No specific comment. See the "All Cases" section for general comments.

Case 3

Genotyping

- No general issues, there was one critical genotyping error for this case.

Interpretation

- Many laboratories did not address the consequence of not finding a pathogenic variant – the patient is still considered at elevated risk and requires monitoring according to their personal and family history. (i.e. a negative report still requires an interpretation)
- Deductions were given for failure to provide adequate details of NGS-based assay limitations such as sensitivity and specificity. This is particularly important if a variant is not identified.
- It is recommended that negative reports include expected diagnostic yield, genes and regions analysed, analytical sensitivity, spectrum of detectable mutations and assay limitations.
- There were four critical interpretation errors for this case. One laboratory reported the benign polymorphic *BRCA2* c.9976A>T p.(Lys3326Ter) variant as pathogenic. There were conflicting results in the report for one laboratory. Some laboratories reported a VUS in *MUTYH* in this sample. The *MUTYH* gene is not associated with HBOC and should not have been tested. Two laboratories were given a critical error for suggesting that this could be the cause of the disease which was very misleading.

Clerical Accuracy

- No specific comment. See the "All Cases" section for general comments.

Professional standards

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

Assessment team

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

Table 1: Assessment Team

Assessors	Location	Role
Norbert Arnold	Germany	Assessor
Luisa Candita	Italy	Assessor
Kai Heinecke	Germany	Assessor
Stacey Hume	Canada	Scheme Organiser
Elizabeth Johnston	UK	Assessor
Ulf Kristoffersson	Sweden	Assessor
Mathis Lepage	France	Assessor
Clemens Müller	Germany	Assessor
Ana Osorio	Spain	Assessor

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Audrey Remenieras	France	Assessor
Rob van der Luijt	Netherlands	Assessor

Appeals

The HBOC panel 2024 Summary Scheme Report (pre-appeals)v1 was published on the 27/06/2024. There were thirty-eight appeals submitted against the marking of the scheme results by twenty-two laboratories. These appeals were reviewed by the members of the scheme assessment team alongside the EMQN team. Sixteen of these appeals were upheld, six appeals were partially upheld, and sixteen 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.

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 sub-contracted 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)[4].

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 2025 EQA, 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 Stacey Hume

Scheme Organiser

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APPENDICES

Rationale for clinical cases

Case 1

This was a male case with no personal history of cancer. Testing was requested due to family history and in relation to risks for any of his offspring. A variant is present in the *ATM* gene for this sample. We expected reports to include information about risk for both the male patient and for offspring.

Case 2

This was a male breast cancer patient with a *BRCA2* splice variant. We expected laboratories to report results with appropriate information relating to risk in the context of a male patient.

Case 3

This was a female individual with breast cancer, and a family history of breast cancer. There is a benign *BRCA2* nonsense variant but no pathogenic variants are present in this sample. We expected reports to include clear limitations of test, particularly test sensitivity. It is not reccomended to include bening variants in clinical reports. However,if the benign 'polymorphic stop' variant was mentioned in a report, is should be clearly stated this is a benign variant. We also expected reports to include appropriate recommendations for next steps for the patient bearing in mind the family history of breast cancer.

Participation

Table 2: Participation data

Participation Details	Number
Number of registrations	205
Number of withdrawals	3
Number of laboratories that did not submit results	6
Total number of participating laboratories	196

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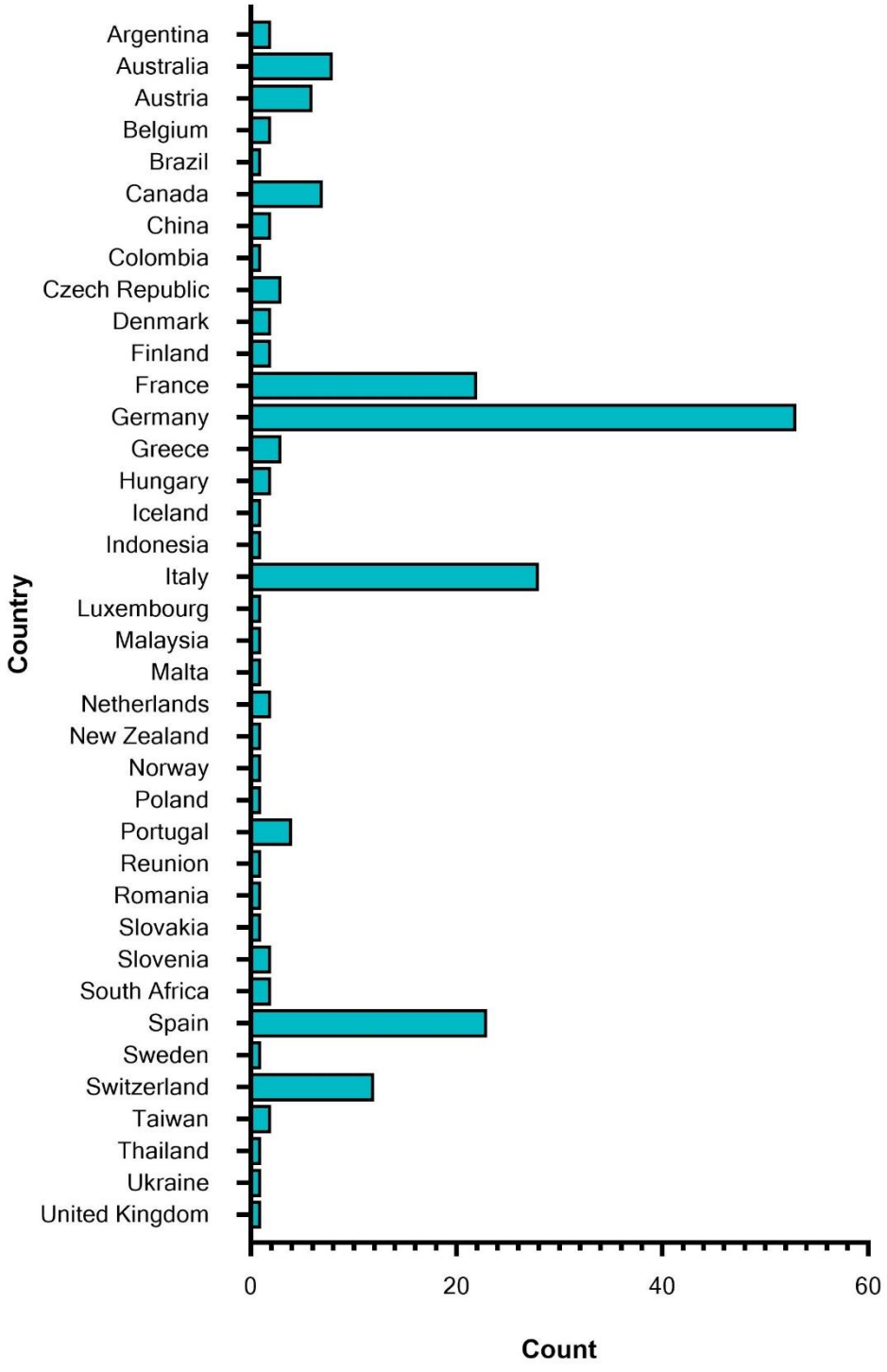
Figure 1: Participating countries



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Samples Provided and Validated Results

The participants received DNA (in TE buffer) extracted from lymphoblastoid cell lines. The genotype of each EQA sample was validated independently using NGS, Sanger sequencing and MLPA, in two different laboratories. Diagnostic requests for the three mock clinical cases were sent together with the samples. 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	Auberon KLEIN	Male	11/01/1970	Auberon KLEIN has no personal history of cancer. However, his family history includes an older brother diagnosed with prostate cancer and two of his four older sisters deceased from breast cancer. Auberon wishes to know if his children are at risk of hereditary breast and ovarian cancer (HBOC)-related tumours so he consents to a full HBOC panel test.	Heterozygous for *ATM* variant NM_000051.4:c.7271T>G p.(Val2424Gly)
2	Aksel OLSEN	Male	05/06/2000	Aksel OLSEN has been recently diagnosed with breast cancer. He has no family history of hereditary breast and ovarian cancer (HBOC)-related tumours. Aksel has children and wishes to know if he has a germline pathogenic variant in any of the HBOC-related genes. He consents to a full HBOC genetic test.	Heterozygous for *BRCA2* variant NC_000013.10(NM_000059.4):c.7977-1G>C p.?
3	Nalini PATIL	Female	01/01/1970	Nalini PATIL has been recently diagnosed with breast cancer. Her mother and two of her mother's sisters developed breast cancer in their 50's and therefore, Nalini consents to a full hereditary breast and ovarian cancer test.	No pathogenic variants

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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 [3,4].

Table 4: EQA Marking Criteria

Case	Category	Criterion	Deduction
All Cases	Genotyping	• Correct result reported	0
		• Critical genotyping error	2
		• Including benign variants in a clinical report is not recommended	0
		• Failure to indicate the 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
		• 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
		• Non-standard transcript used	0.5
		• Transcript missing / incorrect / inconsistent	0.2
		• Transcript version number missing / incorrect / inconsistent	0
		• LRG reference sequences are no longer supported, we advise using MANE transcripts instead.	0
		• Comment with deduction	0.25
		• Comment with deduction	0.5
		• Comment with no deduction	0
		• Not marked	0
		• Testing Failed	0
		• Withdrawn from scheme	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
		• No/Incorrect interpretation of the biological effect of variant	0.5
		• Unrequested testing (Overtesting performed for diagnostic test request)	0.25
		• No indication of pathogenicity of variant detected / incorrect pathogenicity of variant detected	0.5
		• No/insufficient evidence for classification of variant	0.25
		• Insufficient detail regarding variant classification system/evidence used to support classification	0
		• The term “carrier” must not be used for AD disorder	0
		• Failure to complete further testing or refer elsewhere for further testing	0.25
		• 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 state which classes of variants are reported	0.25
		• Failure to state which assay or methodology was used	0.5
		• Failure to report basic QC measures of the NGS test (e.g.- regions captured, mean/median coverage of targets)	0.25

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		• Failure to report the basic details of the bioinformatic pipeline.	0.25
		• Failure to provide adequate description of the clinical targets analysed within the reportable range	0.2
		• Failure to provide capture /amplification kit (and version) and chemistry used for target enrichment and library preparation	0.2
		• Failure to provide adequate details of NGS-based assay limitations eg. Limit of detection (LOD), sensitivity, specificity	0.2
		• Clerical errors causing potential for patient harm e.g. incorrect/inconsistent use of the patient name	1
		• Spelling and typographic errors in the body of the text that changes the meaning of the report	1
		• Comment with deduction	0.25
		• Comment with deduction	0.5
		• Comment with no deduction	0
		• Not marked	0
		• Not marked (due to critical genotyping error)	0
		• Testing Failed	0
		• Withdrawn from scheme	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 sex of patient	0
		• Failure to provide patient identifiers on each page of the report	0.2
		• No description of sample type and/or source of DNA (germline)	0.25
		• 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
		• Reason for referral not restated	0
		• Failure to anonymise report	0
		• Spelling and typographic error in the body of the text that do not change the meaning of the report	0
		• Very long reports; 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 (eg X of Y)	0
		• Clear and concise report	0
		• Not marked	0
		• Not marked (due to critical genotyping error)	0
		• Testing Failed	0
		• Withdrawn from scheme	0
Case 1	Genotyping	• Correct within limitations of the test, there is an ATM variant NM_000051.4:c.7271T>G p.(Val2424Gly) that has not been reported.	0
	Interpretation	• Counselling and/or follow up is relevant but not mentioned in report	0.5
		• Patient should be monitored according to family and personal history - this is not stated	0.5
		• Failure to suggest monitoring programme or indicate patient is at risk of further disease.	0.5
		• Risk to offspring of autosomal recessive disorder should be mentioned	0
		• Failure to test one or more critical genes associated with the clinical referral and to clearly state that the (negative) result is limited in this regard.	1
		• It is recommended that negative reports include expected diagnostic yield, genes and regions analysed, analytical sensitivity, spectrum of detectable mutations and assay limitations	0

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		• Generic (female) report does not apply to this male proband	0.25
Case 2	Interpretation	• 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
		• Generic (female) report does not apply to this male proband	0.25
Case 3	Interpretation	• Patient should be monitored according to family and personal history - this is not stated	0.5
		• Failure to provide scope of the test(s) used i.e. which exons / codons / variants are covered	0.2
		• Failure to test one or more critical genes associated with the clinical referral and to clearly state that the (negative) result is limited in this regard.	1
		• It is recommended that negative reports include expected diagnostic yield, genes and regions analysed, analytical sensitivity, spectrum of detectable mutations and assay limitations	0

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Results: summary statistics

The mean scores for genotyping/analytical, interpretation, clerical accuracy and the total mean 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)	2.0 (0.04)	1.98 (0.08)	1.99 (0.15)
	Median (SD)	2.0 (0.04)	2.0 (0.08)	2.0 (0.15)
Interpretation	Mean (SD)	1.82 (0.34)	1.81 (0.31)	1.63 (0.42)
	Median (SD)	2.0 (0.34)	2.0 (0.31)	1.75 (0.42)
Patient Identifiers & Clerical Accuracy	Mean (SD)	1.92 (0.16)	1.92 (0.18)	1.93 (0.15)
	Median (SD)	2.0 (0.16)	2.0 (0.18)	2.0 (0.15)

There was one critical genotyping errors made by one laboratory (1/196, 0.51%) (see Table 6). Five critical interpretation errors were reported by five laboratories (5/196, 3.1%). 190 laboratories (190/196, 96.94%) achieved a satisfactory result.

Table 6: Critical Genotyping Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases completed	195	196	195	586
Number of laboratories with full marks	185	178	185	548
Number of critical errors	0	0	1	1
Error rate (%)	0	0	0.51	0.17

Table 7: Critical Interpretation Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases assessed	187	189	187	563
Number of laboratories with full marks	119	113	69	301
Number of critical errors	0	1	4	5
Error rate (%)	0	0.53	2.14	0.89

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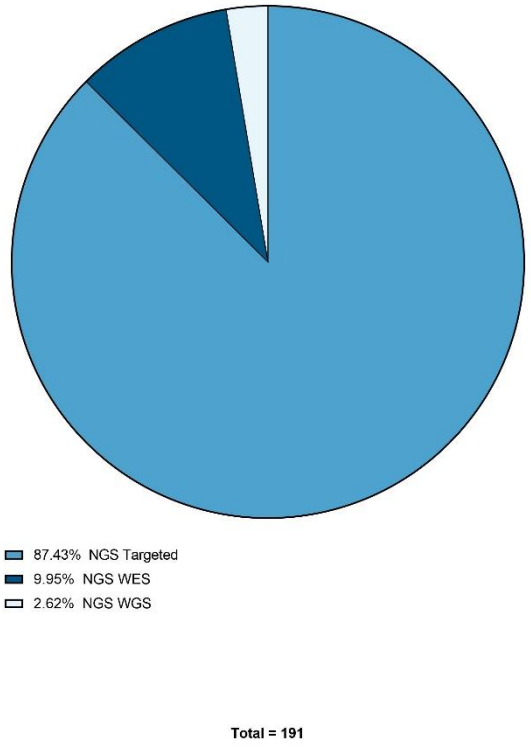
Results: Critical genotyping Errors Summary

Table 8 below shows a breakdown of the critical genotyping errors made by laboratories that participated in this EQA scheme.

Table 8: Summary of critical errors made in this EQA scheme

Case	Error	Description	Number of laboratories
1	False negative	Failure to report *ATM* variant NM_000051.4:c.7271T>G p.(Val2424Gly)	1
3	False positive	False positive for NM_001042492.3(*NF1*):c.3198A>T (p.Arg1066Ser)	1

Results: Methodology used



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Amendments to this summary EQA report

Version	Page	Section	Change	Published
1	**6**	**Appeals**	The HBOC PANEL 2024 Summary scheme report (pre-appeals)v1 was updated with outcome of appeals to create HBOC PANEL 2024 Summary scheme report (post-appeals)v1	22th August 2024
2				
3				

Authorisation

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

Dr. Simon Patton on 22th August 2024

CEO

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