ESR1 testing in Breast Cancer [Plasma] Pilot EQA 2024

Summary
Scheme Report

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Table of contents

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09 April 2025

Dear Colleague,

This external quality assessment (EQA), *ESR1* in Breast Cancer (Plasma) 2024 is a pilot 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 "*ESR1* testing in Breast Cancer [Plasma]" EQA.

EQA design and purpose

The aim of this pilot 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 *ESR1* testing in breast cancer cfDNA [Plasma] 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 clinically significant *ESR1* variants in circulating free DNA (cfDNA).
- 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

General

- A total of 30 laboratories registered to participate in the EQA scheme with 29 laboratories submitting results. One laboratory withdrew from participation.
- Next Generation Sequencing (NGS) was utilised by 62.1% (18/29) of participants as their primary method, 27.6% (8/29) used Digital droplet PCR (ddPCR), 10.3% (3/29) of laboratories using Real-Time / Fluorescent PCR kits, and 3.5% (1/29) used Sanger sequencing (see Table 7 for more detail). Sanger sequencing is not sufficiently sensitive for detection of variants in cfDNA, and we strongly advise use of a more appropriate method.

All Cases

Genotyping

- There were no critical genotyping errors reported by any of the 29 participating laboratories. The mean genotyping score was 1.98.
- Overall, use of HGVS nomenclature was good. There were some minor observations around incorrect use of brackets around the protein change or not using brackets at all.
- Variants should be described in terms of the nucleotide change and the predicted amino acid change. Three-letter amino acid codes are preferred.
- 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[1]. Support for Locus Reference

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Genomic (LRG) reference sequences has been discontinued. While use of LRG reference sequences is still acceptable, 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.

Interpretation

- This EQA was designed to assess the ability of laboratories to test for clinically relevant *ESR1* variants in cfDNA samples and interpret the results in the context of the clinical referral to guide treatment with selective estrogen receptor degraders (SERD).
- A few laboratories failed to report the limit of detection (LOD) of their assays.

Clerical Accuracy

- Generally, the level of clerical accuracy reporting was high with an average score of 1.81. However, the following observations were made.
- The full reason for referral was not restated in some clinical reports. It may contain important clinical information and gives context to the reader.
- If the report has multiple pages, a minimum of two patient identifiers must be present on all pages to maintain the integrity of the report.
- It is good practice to include date of sample receipt and reporting in the report. In many instances only one date was provided.
- In some reports pagination was not provided in the correct format. Pagination omissions and errors can cause inadvertent loss of critical data from a forwarded report or physical copy. Including pagination, e.g., Page 1 of 1, indicates the expected report length.

Case 1

Genotyping

- Case 1 had an *ESR1* variant NM_000125.4:c.1138G>C p.(Glu380Gln) at a variant allele frequency (VAF) of 16.1%. It did not have any other actionable variants.
- All laboratories correctly reported the c.1138G>C p.(Glu380Gln) variant with 27 out of the 29 laboratories recieving full marks.
- The average VAF reported was 23.2% (Figure 5)

Interpretation

- There were no critical interpretation errors reported in case 1 and the mean score was 1.76.
- Deductions were applied where laboratories had failed to mention SERDs in relation to clinical referral. Clinical interpretations must be tailored to the individual referral reason, the patient tested, and the specific results obtained.

Case 2

Genotyping

- Case 2 had two *ESR1* variants, NM_000125.4:c.1613A>G p.(Asp538Gly) and NM_000125.4:c.1610A>C p.(Tyr537Ser) at VAFs of 12.1% and 3.1% respectively.
- There were no critical genotyping errors reported in this case. The mean score was 1.96 with 26 out of the 29 laboratories receiving full marks.
- The average VAFs reported were 16.7% and 3.4%, respectively. (Figure 6)

Interpretation

- There were no critical interpretation errors reported in case 2 and the mean score was 1.89.
- Deductions applied in case 2 in the clinical interpretation category were the same as in case 1.

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Case 3

Genotyping

- Case three did not have any pathogenic variants in *ESR1*.
- The mean score in this case was 1.99 with 28 out of 29 laboratories receiving full marks.

Interpretation

- There were no critical interpretation errors reported in case 3.
- The mean score in the interpretation category was 1.68.
- The main deduction applied in this case were due to laboratories inappropriately interpreting the absence of an actionable variant. For instance, stating that patient is unlikely to benefit from SERDs if no variant was detected. The possibility that there may not be enough circulating tumour DNA (ctDNA) in the sample to detect a variant should be stated on the report.
- Laboratories also received deductions for failing to state that a repeat sample/or that serial testing should be recommended.

Professional standards

Laboratories are assessed against the guidelines and relevant peer reviewed literature currently available references[2]. 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
Dr James Beasley	United Kingdom	Scheme assessor
Dr Aliki Ntzifa	Greece	Scheme assessor
Paula Proszek	United Kingdom	Scheme assessor
Arfa Maqsood	United Kingdom	Scheme organiser
Victoria Williams	United Kingdom	Scheme organiser

Appeals

The marking is not subject to appeal as this is a pilot EQA scheme with no associated poor performance. However, if you wish to comment on your report or contact the relevant EQA provider, you can do this by email (office@emqn.org). Please include your laboratory account number, the name and year of the EQA scheme and details of the case on which you wish to comment/enquire.

Confidentiality

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

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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 (ISO15189:2022 REQ 6.8.2 and REQ 7.4.1.7)[2].

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 registration is opened until 31st March 2025 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,

Arfa Maqsood

Scheme Organiser

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APPENDICES

Rationale for clinical cases

Case 1, 2 and 3

ESR1 variants are acquired during treatment and found in up to 40% of patients with metastatic hormone receptor-positive breast cancer who have received endocrine therapy[3].

In light of new oestrogen receptor antagonist data, recently updated oncology guidelines recommend routine testing for *ESR1* variants at disease recurrence or upon disease progression on endocrine therapy in patients with ER-positive, HER2-negative metastatic breast cancer[4].

Case 1

- Material with an activating *ESR1* hotspot variant.
- Variant present at a VAF that should be detectable by most test methodologies.
- To determine how participants would interpret it in the context of SERD therapy.

Case 2

- Material with multiple activating *ESR1* hotspot variants.
- Variants present at a VAF that should be detectable by most test methodologies.
- To determine how participants would interpret it in the context of SERD therapy.

Case 3

- Material with no activating variants.
- Interpretation expected to discuss the sensitivity of cfDNA analysis and recommendations for a repeat sample or serial testing

Participation

Table 1: Participation data

Participation Details	Number
Number of registrations	30
Number of withdrawals	1
Number of laboratories that did not submit results	0
Total number of participating laboratories	29

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Figure 4: Participating countries

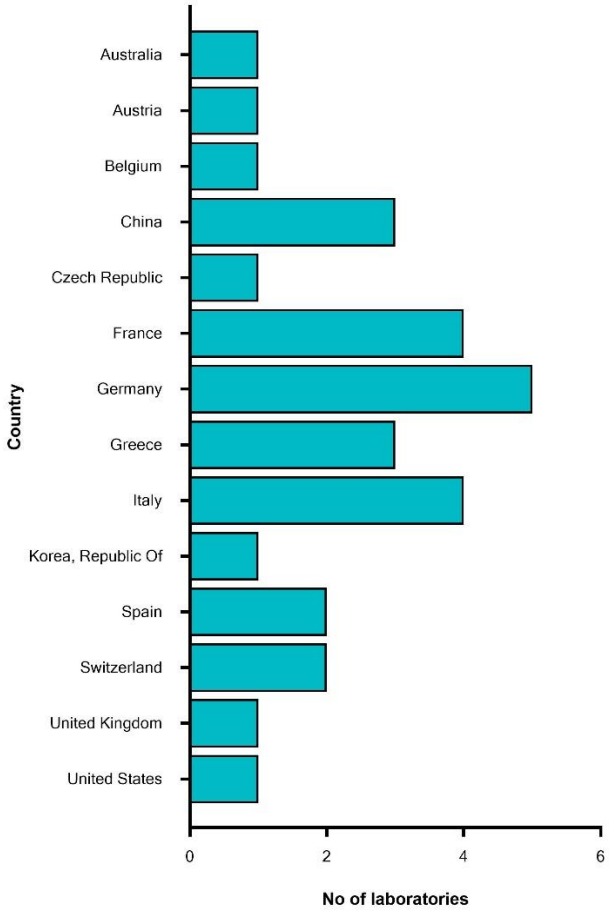

Figure 5: Variant Allele Frequencies reported in for case 1 vs consensus data from ddPCR validation

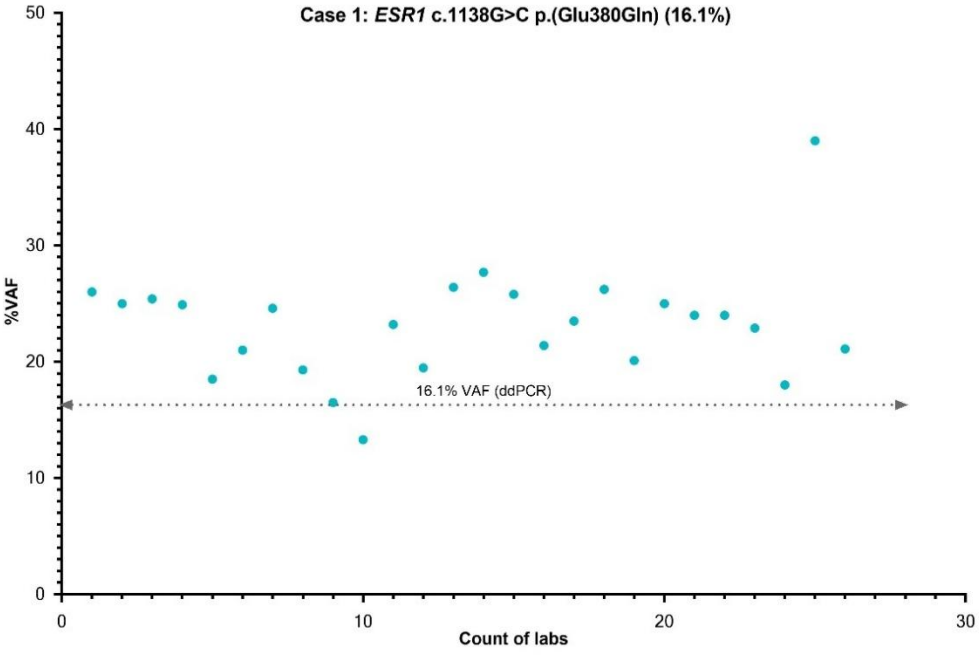

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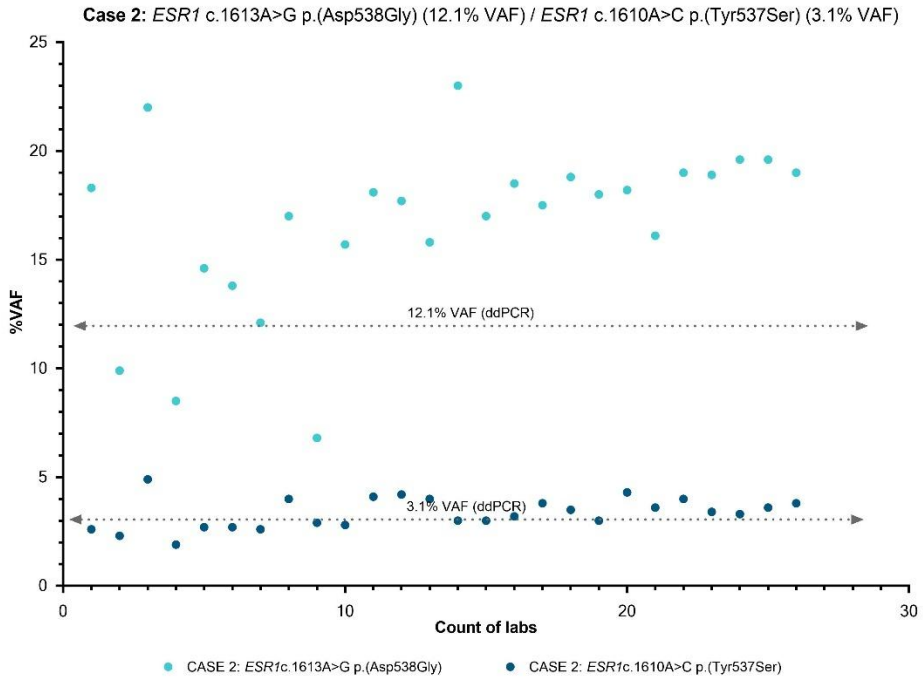
Figure 6: Variant Allele Frequencies reported in for case 2 vs consensus data from ddPCR validation



Samples Provided and Validated Results

The participants received 3 commercially manufactured cfDNA samples containing DNA of human origin from cell lines containing *ESR1* variants blended with DNA depleted plasma. The genotype of each EQA sample was validated independently using NGS, in 2 different external validated laboratories. Diagnostic requests for the 3 mock clinical cases were sent together with the samples. The VAFs for cases 1-2 have been validated by the manufacturer using ddPCR. The validated results are shown in Table 2.

Table 2: EQA Sample details and validated results

Case	Name	Sex	Date of Birth (dob)	Referral Reasons	Validated Result
1	Gelaz BEDAD	F	04/09/1959	Gelaz BEDAD was first diagnosed with ER+/HER2- breast cancer at the age of 50. She underwent whole breast irradiation followed by adjuvant endocrine therapy for 5 years. At 62 years of age, she presented with chest pain during exertion and shortness of breath. A PET scan and core biopsy from a pleural lesion confirmed metastases of breast origin. Gelaz was treated with Aromatase and CDK4/6 inhibitors for 24 months at which point further scans showed pleural progression accompanied by pulmonary nodules and a new 1.5 cm liver lesion. Testing of plasma for *ESR1* variants has been	NM_000125.4: c.1138G>C p.(Glu380Gln) (16.1%)

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				requested to aid clinical decision making.	
2	Evelyn JENKINS	F	09/03/1961	Evelyn JENKINS was first diagnosed with grade 3, ER+/HER2- breast cancer at the age of 51, and was treated with breast conserving surgery, adjuvant radiotherapy and endocrine therapy for 5 years. At age 62, metastases of breast origin were revealed by a PET scan. A liver biopsy was negative for any clinically actionable variants. Evelyn received treatment with Aromatase inhibitor and a CDK4/6 inhibitor. However, 12 months into her treatment she presented with a general malaise and underwent a CT scan that showed enlargement of the liver lesions and new bone lesions. Testing of plasma for *ESR1* variants has been requested to aid clinical decision making.	NM_000125.4 : c.1613A>G p.(Asp538Gly) NM_000125.4: c.1610A>C p.(Tyr537Ser)(12.1% and 3.1%)
3	Helene WAISMANN	F	22/10/1962	Helene WAISMANN has a history of ER+/HER2- invasive ductal lobular breast cancer and underwent surgery. She was treated with post-operative chemotherapy, radiotherapy and adjuvant hormone treatment for 5 years. She recently presented with hip pain and pelvic bone lesions. NGS panel testing on a bone biopsy did not identify any clinically actionable variants. Treatment with an Aromatase inhibitor in combination with a CDK4/6 inhibitor was initiated. 16 months into the treatment, a routine scan identified additional smaller lesions. Testing of plasma for *ESR1* variants has been requested to aid clinical decision making.	No variants 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[2,5-6]

Table 3: EQA Marking Criteria

Category	Category	Criterion	Deduction
All Cases	Genotyping	• Correct result reported	0.0
		• Critical genotyping error	2.0
		• Transcript missing / incorrect / inconsistent	0.2
		• Transcript version number missing/incorrect/inconsistent	0.0
		• LRG reference sequences are no longer generated or updated. We recommend you change to MANE Select or MANE Plus Clinical	0.0
		• Test failure giving no result for the sample and did not state that a repeat sample should be requested	0.5
		• Test failure giving no result for the sample and stated that a repeat sample should be requested	0.0
		• Comment with deduction	0.2
		Comment with deduction	0.5
		• Comment with deduction	1.0
		• Comment only	0.0
		• Not marked	0.0
		• Withdrawn from scheme	0.0
	Interpretation	• All essential interpretative elements provided. No deductions.	0.0
		• No clinical interpretation	2.0
		• Critical interpretation error	1.5
		• No mention of Selective estrogen receptor degraders (SERD) therapy	1.0
		• No/insufficient information about the methodology performed	0.5
		• No patient specific clinical interpretation given	0.5
		• Misleading interpretive comment	1.0
		• Clerical error(s) causing potential for patient harm e.g. incorrect or inconsistent use of patient name in the body of the report	1.0
		• Comment only	0.0
		• Comment with deduction	0.2
		• Comment with deduction	0.5
		• Comment with deduction	1.0
		• Not marked	0.0
		• Not marked (due to critical genotyping error)	0.0
		• Withdrawn from scheme	0.0
	Clerical Accuracy	• All essential patient identifiers present and no significant clerical errors	0.0
		• The patient's name has small spelling error	1.0
		• Date of birth (dob) incorrect/missing	0.5
		• Incorrect or missing sex of patient	0.0
		• The full reason for referral should be included in the report	0.2
		• Failure to provide sample identifiers (REF and/or LOT number)	0.2
		• The sample type should be given on the report	0.2
		• The sample type is incorrect (eg., FFPE instead of plasma)	0.2
		• Failure to provide the dates of sample receipt/testing or reporting	0.2
		• Failure to provide patient identifiers on each page of the report	0.2
		• There is no evidence that the report was authorised i.e. no indication report signed by two people	0.0
		• Incorrect pagination (use if states Page 2 of 1, for example)	0.2

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		• Failure to provide correct pagination e.g. pagination missing or only states Page 1 instead of Page 1 of 1 etc.	0.2
		• The report should be anonymised	0.0
		• The essential clinically relevant information is 'lost' in this long report. Consideration should be given to reducing the length of the reports	0.0
		• Comment only	0.0
		• Comment with deduction	0.2
		• Comment with deduction	0.5
		• Comment with deduction	1.0
		• Clear and concise report	0.0
		• Not marked	0.0
		• Not marked (due to critical genotyping error)	0.0
		• Withdrawn from scheme	0.0
Case 1	Genotyping	• HGVS nomenclature not used / incorrect HGVS nomenclature	0.5
		• No indication of pathogenicity of variant detected / incorrect pathogenicity of variant detected	0.5
		• Minor HGVS error e.g. missing brackets around the protein or p. inside the brackets	0.2
		• Major HGVS error (Genotype mis-positioned or mis-called egg, incorrect base/amino acid detected)	0.5
	Interpretation	• No/insufficient information about the limitations of the test performed (LOD). No deduction made as a pathogenic variant has been identified	0.0
		• No/Insufficient details of the scope of the test. No deduction made as a pathogenic variant has been identified	0.0
Case 2	Genotyping	• HGVS nomenclature not used / incorrect HGVS nomenclature	0.5
		• No indication of pathogenicity of variant detected / incorrect pathogenicity of variant detected	0.5
		• Minor HGVS error e.g. missing brackets around the protein or p. inside the brackets	0.2
		• Major HGVS error (Genotype mis-positioned or mis-called e.g., incorrect base/amino acid detected)	0.5
	Interpretation	• No/insufficient information about the limitations of the test performed (LOD). No deduction made as a pathogenic variant has been identified	0.0
		• No/Insufficient details of the scope of the test. No deduction made as a pathogenic variant has been identified	0.0
Case 3	Genotyping	• Correct result within limitations of the test performed (i.e. assay used does not detect the variant present)	0.0
	Interpretation	• Over / inappropriate interpretation of a negative (or normal) result using cfDNA. For example, advising that the absence of the mutation indicates that the patient would be unlikely to benefit from SERDs. The report should state that it is possible that the levels of circulating tumour DNA in this sample may be too low to detect a potential mutation	0.5
		• Failure to provide adequate details of assay limitations e.g. Limit of detection (LOD), sensitivity, specificity	0.2
		• Failure to provide scope of the test(s) used i.e. which exons / codons / variants are covered	0.2
		• The report should recommend that a repeat sample should be sent for testing, or serial testing should be recommended	0.5

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 4. A summary of the number of critical errors per case is provided in Tables 5 & 6.

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

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Table 4: Mean Scores

Category		Case 1	Case 2	Case 3
Genotyping	Mean (SD)	1.98	1.96	1.99
	Median (SD)	2.0	2.0	2.0
Interpretation	Mean (SD)	1.76	1.89	1.68
	Median (SD)	2.0	2.0	2.0
Patient Identifiers & Clerical Accuracy	Mean (SD)	1.82	1.84	1.78
	Median (SD)	2.0	2.0	2.0

There were no critical genotyping or critical interpretation made by any of the laboratories (see Table 6). Therefore, all laboratories achieved a satisfactory result.

Table 5: Critical Genotyping Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases completed	29	29	29	87
Number of laboratories with full marks	27	26	28	81
Number of critical errors	0	0	0	0
Error rate (%)	0	0	0	0

Table 6: Critical Interpretation Errors

Category	Case 1	Case 2	Case 3	Total
Number of cases assessed	23	23	23	69
Number of laboratories with full marks	18	19	13	50
Number of critical errors	0	0	0	0
Error rate (%)	0	0	0	0

Results: Methodology used

Table 7.

Commercial kit names as provided by participants

Methodology	Count
NGS	**18**
Agilent	**1**
SureSelect XT HS2 DNA kit	1
ArcherDX (IDT)	**3**
Archer LIQUIDPlex™ Universal Solid Tumor Panel	3
IMB dx	**1**
AlphaLiquid® 100	1
Genes2Me	**1**
ctDNA Breast Panel CE, IVD	1
GenePlus	**1**
Oncology Multi-Gene Variant Assay	1
Hedera Dx	**1**
Hedera Profiling 2 ctDNA test panel	1
In house design	**1**
Thermo Fisher Scientific	**4**
Oncomine™ Breast cfDNA Research Assay v2	2

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Oncomine™ Precision Assay (Genexus)	2
Roche Diagnostics	**1**
KAPA HyperPlus	1
Sysmex Inostics	**1**
Plasma-SeqSensei™ Breast Cancer IVD Kit	1
Other	**3**
Real-Time/ Quantitative-PCR	**3**
Apis Assay Technologies Ltd	**1**
Apis ESR1 Mutation Kit	1
BioTechne Corporation	**1**
Quantidex Qpcr ESR1 exoMuation Kit	1
Diatech Pharmacogenetics	**1**

EasyPGX® ready *ESR1*	1
Digital Droplet PCR	**6**
Bio-Rad	**3**
ddPLEX ESR1 Mutation Detection Kit	3
Thermo-Fisher Scientific	**1**
Absolute Q Digital PCR Assay	1
Stilla Technologies	**1**
ESR1 (17 mutations) Crystal Digital PCR® Assay	1
In house design	**1**
Sanger sequencing	**1**
Thermo Fisher	**1**

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Amendments to this summary EQA report

Version	Page	Section	Change	Published
1	-	-	None	31st March 2025
2				
3				

Authorisation

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

Dr. Simon Patton on 31st March 2025

CEO

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