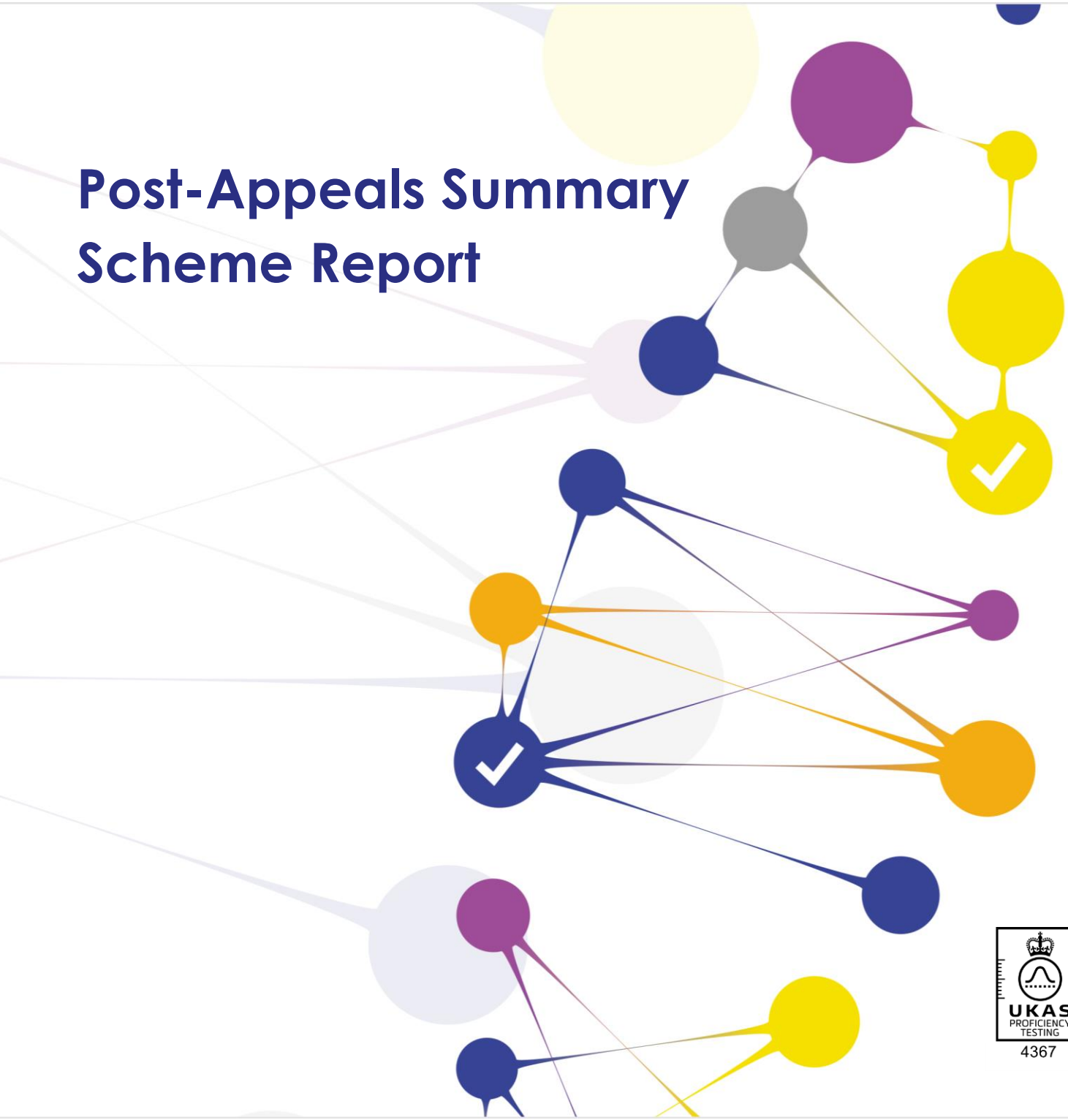
Y-Chromosome Microdeletions (AZF) EQA 2023

Post-Appeals Summary Scheme Report



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Dear Colleague,

This external quality assessment (EQA), Y-Chromosome microdeletions (AZF) 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 2023 "AZF" 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 AZF 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 AZF microdeletions,
- 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

Case	Category	Comments

All cases	*Genotyping*	• Nine critical errors have been awarded, in both case one and case two of the EQA scheme, to the same nine laboratories. For both cases the laboratories involved made identical errors in their reporting.
		• EMQN reminds laboratories that their submissions must represent the work from their own laboratory, and that it is the responsibility of each laboratory to inform EMQN of any sub-contracted activities which form part of their testing process. Our terms and conditions state:
		  • 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 (ISO 15189 REQ 4.5.2 and REQ 5.8.3)[3].
		  • Laboratories which have been found to have colluded and/or falsified results will be excluded from participating in future EQA schemes and where necessary, the relevant competent authority will be notified.
		  • In cases where collusion is strongly suspected, EMQN reserves the right to withhold the certificate of participation for the specified scheme year from the relevant laboratories.
		• Two types of positive controls should be included in the test (and their results listed in the report): one mapping either to an autosome or, preferably, to the X chromosome; the other to Yp (in the EAA/EMQN Guidelines we recommended *ZFX*/*ZFY* and *SRY*, respectively)[1].
		• The result of all markers that are essential to determine the genotype must be reported (present / absent).

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		• Extension analysis is mandatory in all cases where a deletion has been identified. Only through extension analysis can the clinical consequences of the detected variants be fully determined. • In cases where commercial kits are used, the corresponding version number(s) should be provided and the results for all individual markers must also be reported.
	Interpretation	• The genotype - phenotype correlation must be clearly stated in all cases (i.e., irrespective of a deletion being identified or not). • Complete restatement of the reason for referral is an important component of the report and required by best practice guidance[4].
	Clerical Accuracy	• Reports should be one page only and preferably signed by two specialists. This year, a significant number of laboratories (34%) provided excessively long reports for case 2.
Case 1	*Genotyping*	• This was a Y chromosome without a complete AZF deletion BUT with a gr/gr (partial AZFc) deletion.
	Interpretation	• Karyotyping should be advised (irrespective of the detection of the partial deletion). • The gr/gr deletion is a population-dependent risk factor of spermatogenic impairment. Thus, mentioning TESE in this context is misleading. • If the laboratory did not test for gr/gr deletion, the result should be interpreted as "absence of complete AZF deletions" and it should be stated that the "genetic cause for the phenotype was not identified". • *CFTR* analysis should not be recommended in the context of AZF diagnostic tests. This analysis should be requested by the clinician in selected cases e.g. Congenital absence of vas deferens (CAVD).
	Clerical Accuracy	• A number of laboratories, who also had genotyping errors, had a spelling error in the name of the patient.
Case 2	*Genotyping*	• This was an atypical complete AZFa deletion (proximal breakpoint defined by the presence of sY82*and of sY83*, and the absence of sY1064). • The presence of sY83 in this complete AZFa deletion pattern is compatible with the previously reported variability in both the proximal and distal breakpoints of this rearrangement[5]. Of note, this variability indicates that sY83 and sY1064, both recommended in the current version of the EAA/EMQN guidelines as suitable markers for the deletion extension step, can in fact lead to different results depending on the type of proximal breakpoint. The expected course of action in this case would be for laboratories to confirm the atypical sY83 positive result with the equally recommended sY1064 marker (always absent in complete AZFa deletions). To simplify matters, this situation will be addressed in a revised version of the guidelines – to be published later this year – where only sY1064 marker will be recommended, alongside sY82 (positive marker), for the deletion extension analysis. Considering the significant challenge posed by this case, we decided not to penalize any laboratories reporting the deletion as partial based on the presence of the sY83 marker. However, we stress that the AZFa genes map to the distal end of the region, hence even if only sY83 was tested, the laboratories should have concluded, based on the absence of both sY84 and sY86 (first round analysis) as well as on the result of the distal breakpoint markers, that the deletion did in fact remove both genes, and interpret the rearrangement accordingly in terms of genotype-phenotype correlation and prediction of TESE outcome.
	Interpretation	• In cases where only the sY83 marker was tested and found to be present, the deletion should have not been reported as complete; a second proximal marker (absent) should also have been tested (sY1064; is deleted in this patient); 27 (20%) laboratories tested only the sY83 marker and concluded the deletion was complete; whereas 28 (21%) concluded the deletion was partial; six laboratories(4%) tested both sY83 and sY1064

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		markers and concluded there was a partial deletion; a comment was made in all cases. • The prognosis for TESE with a complete AZFa deletion is virtually zero. There should be a clear statement of this included in the report.
	Clerical Accuracy	• No specific comments. See the "all case" section for general comments.
Case 3	*Genotyping*	• This was a complete, interstitial AZFc deletion (b2/b4). • Identification of this variant as a complete AZFc deletion (or b2/b4) is essential in order to distinguish it from partial AZFc deletions.
	Interpretation	• Cause–effect relationship must be clearly stated regarding the patient's phenotype, as well as the average likelihood of finding sperm in a testicular biopsy (around 50%). • Transmission of the deletion to all sons should be acknowledged. • The testing of male relatives should be recommended. • Karyotyping should always be advised, regardless of the deletion being interstitial or terminal.
	Clerical Accuracy	• No specific comments. See the "all case" section for general comments.

Professional standards

Laboratories are assessed against the guidelines, relevant peer reviewed literature and currently available references. Other guidelines against which laboratory reports are assessed may include the international nomenclature HGVS[2] and ISO standards (ISO15189)[3].

Assessment team

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

Table 1: Assessment Team

Assessors	Location	Role
Csilla Krausz	Italy	Scheme Organiser
Paulo Navarro Costa	Portugal	Assessor
Frank Tuettelmann	Germany	Scheme Organiser
Martina Wilke	Netherlands	Assessor

Appeals

The AZF 2023 scheme summary report (v1) was published on the 11/07/2023. There were 10 appeals submitted against our marking of the scheme results by four laboratories. These appeals were reviewed by the members of the scheme assessment team alongside the EMQN team. Six of these appeals were upheld, and four 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.

Confidentiality

Details of our confidentiality policies can be found here: See https://www.emqn.org/participating-in-eqa/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 (ISO 15189 REQ 4.5.2 and REQ 5.8.3)[3].

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 2024 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. Martina Wilke, Dr. Paulo Navarro-Costa, Prof. Frank Tüttelmann and Dr. Csilla Krausz

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APPENDICES

1. Rationale for clinical cases

Case 1:

When analysing for AZF deletions, a negative result with the standard markers will be most common but warrants good interpretation and especially advice for further testing.
Since a growing number of laboratories test also for gr/gr deletion (partial AZFc), we included this sample to evaluate whether those laboratories which have included gr/gr markers in their routine practice, are able to correctly interpret their finding.

Case 2:

AZFa deletions are the rarest type of deletions, however their clinical impact is relevant since in case of complete AZFa deletion the probability of finding spermatozoa in virtually zero.

Case 3:

AZFc deletions are the most common and, thus, most frequently detected.

2. Participation

Table 2: Participation data

Participation Details	*Number*
Number of registrations	149
Number of withdrawals	3
Number of laboratories that did not submit results	2
Total number of participating laboratories	144

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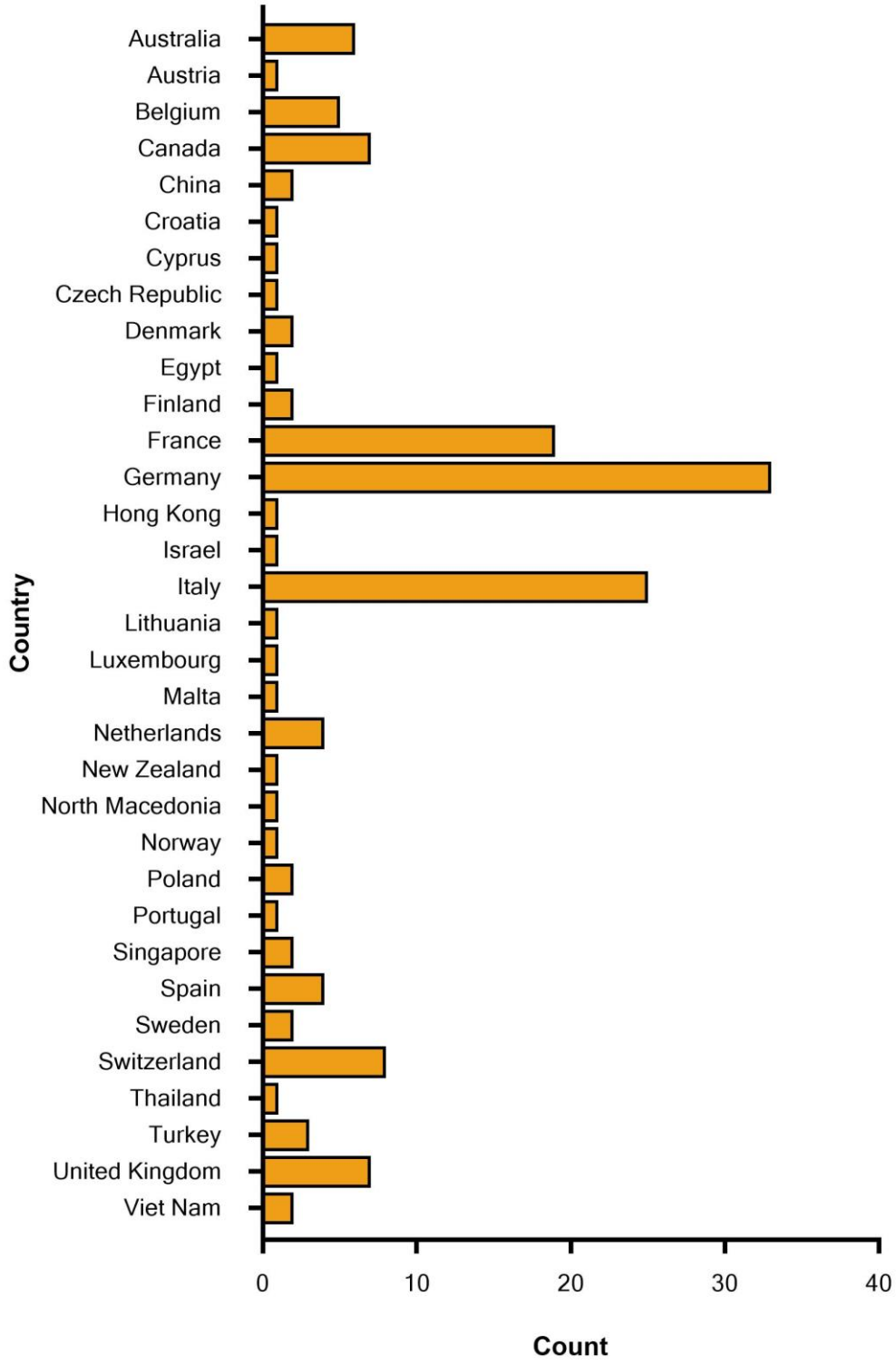
Figure 1: Participating countries



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3. Samples Provided and Validated Results

The participants received DNA (in TE buffer) extracted from a lymphoblastoid cell line. The genotype of each EQA sample was validated independently by PCR of relevant STS loci on the Y chromosome, 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	Francesco COLOMBINI	M	08/08/1998	Francesco has a clinical diagnosis of severe oligozoospermia. AZF analysis is requested as part of the diagnostic work-up for the couple's infertility.	No complete AZF deletion; gr/gr deletion.
2	Krisztian KEPESI	M	06/12/1988	Krisztian has a clinical diagnosis of azoospermia. AZF analysis is requested in preparation for TESE/ICSI.	Complete AZFa deletion; non-classical proximal breakpoint.
3	Frederick LIU	M	22/04/1986	Frederick has a clinical diagnosis of azoospermia. AZF analysis is requested in preparation for TESE/ICSI	Complete AZFc deletion (b2/b4).

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4. 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 [2,3].

Table 4: EQA Marking Criteria

Case	Category	Criterion	Deduction
All Cases	Genotyping	Correct result reported	0
		Critical genotyping error	2
		One marker incorrect but deletion correctly identified	1
		Only one marker tested for each AZF region	0.5
		Result for each individual marker not shown (present/absent)	0.25
		Alternative methodology and the list of markers not fully clarified	0.25
		Control markers not reported (SRY and/or ZFX/ZFY missing)	0.25
		Comment: ZFX/Y marker nomenclature incorrect	0
		Comment: Other than PCR plus minus method	0
		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
		Counselling and/or follow up is relevant but not mentioned in report.	0.5
		Failure to state which assay / methodology was used	0.5
		Kit version number missing e.g. MLPA	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
		Biological interpretation incorrect (either a complete AZF microdeletion was ruled out or a gr/gr deletion was detected)	0.5
		Comment: Cause-effect relationship not recognised (cause for severe oligozoospermia not identified)	0
		Comment: Unnecessary testing advised (e.g. CAVD)	0
		Comment with deduction	0.25
		Comment with deduction	0.5
		Not marked	0
		Not marked (due to critical genotyping error)	0
		Withdrawn from scheme	0
		Test Failed	0
	Patient Identifiers and 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
		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

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		Errors in sample batch no. or not 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. X of Y)	0
		Clear and concise report	0
		Not marked	0
		Not marked (due to critical genotyping error)	0
		Test Failed	0
		Withdrawn from scheme	0
Case 1	Genotyping	Comment: The lab tested for gr/gr deletion	0
	Interpretation	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
		Comment: Cause-effect relationship not recognised (cause for severe oligozoospermia not identified)	0
		Cause-effect relationship not recognised (risk factor for severe oligozoospermia identified)	0.5
		Comment: Testing for male relatives should be recommended	0
		Comment: Karyotyping should be recommended	0
		Comment: Transmission to sons should be stated	0
Case 2	Genotyping	No extension analysis (neither acknowledged nor referred)	0.5
		Extension analysis incorrect (one or more markers)	0.5
		Extension analysis not performed according to guidelines, but tested for additional markers (e.g. in genes)	0.25
		Comment: Extension analysis not performed, but acknowledged	0
	Interpretation	Comment: The lab tested for only sY83 and concluded partial deletion.	0
		Cause-effect relationship not recognised (cause of azoospermia identified)	0.5
		Prognostic value for TESE/ICSI not recognised or not correct	0.5
		Comment: The lab tested for only sY83 and concluded complete deletion	0
		Comment: The lab tested for sY83 and sY1064 and concluded partial deletion	0
		Comment: The lab tested for sY83 and sY1064 and concluded complete deletion	0
Case 3	Genotyping	No extension analysis (neither acknowledged nor referred)	0.5
		Extension analysis incorrect (one or more markers)	0.5
		Extension analysis not performed according to guidelines, but tested for additional markers (e.g. in genes)	0.25
		Comment: Extension analysis not performed, but acknowledged	0
	Interpretation	Cause-effect relationship not recognised (cause of azoospermia identified)	0.5
		Comment: Testing for male relatives should be recommended	0
		Comment: Karyotyping should be recommended	0
		Prognostic value for TESE/ICSI not recognised or not correct	0.5
		Comment: Transmission to sons should be stated	0

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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 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)	1.85 (0.49)	1.8 (0.5)	1.88 (0.26)
	Median (SD)	2.0 (0.49)	2.0 (0.5)	2.0 (0.26)
Interpretation	Mean (SD)	1.9 (0.25)	1.94 (0.21)	1.87 (0.32)
	Median (SD)	2.0 (0.25)	2.0 (0.21)	2.0 (0.32)
Patient Identifiers & Clerical Accuracy	Mean (SD)	2.0 (0.0)	2.0 (0.0)	1.99 (0.06)
	Median (SD)	2.0 (0.0)	2.0 (0.0)	2.0 (0.06)

Table 6: Critical Genotyping Errors

Category	Case 1	Case 2	Case 3	Totals
Number of cases completed	144	144	144	432
Number of labs with full marks	121	110	114	345
Number of critical errors	9	9	0	18
Error rate (%)	6.25	6.25	0	4.16

Table 7: Critical Interpretation Errors

Category	Case 1	Case 2	Case 3	Totals
Number of cases assessed	135	136	145	416
Number of labs with full marks	108	120	119	347
Number of critical errors	1	0	0	1
Error rate (%)	0.74	0	0	0.24

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6. Results: Critical genotyping Errors Summary

Nine laboratories made 18 critical genotyping errors. Please note this table shows errors associated with a particular test methodology as defined by the scope and LODs declared by the participant laboratories. The names of assays are also as provided by participants.

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

Case	False positive results			False negative results		
	Count	Reported result (count)	Method	Count	Missed result (Count)	Method
1	9	AZFc DEL: Yq11.21-Yq11.21 (21880096_57197186) instead of No complete AZF deletion; gr/gr deletion.	Clinical Exome NGS Sequencing	0	n/a	n/a
2	9	AZFa DEL: Yq11.21-Yq11.21 (12857305_12859676) AZFc DEL: Yq11.21-Yq11.21 (21880096_57197186) instead of Complete AZFa deletion; non-classical proximal breakpoint.	Clinical Exome NGS Sequencing	0	n/a	n/a

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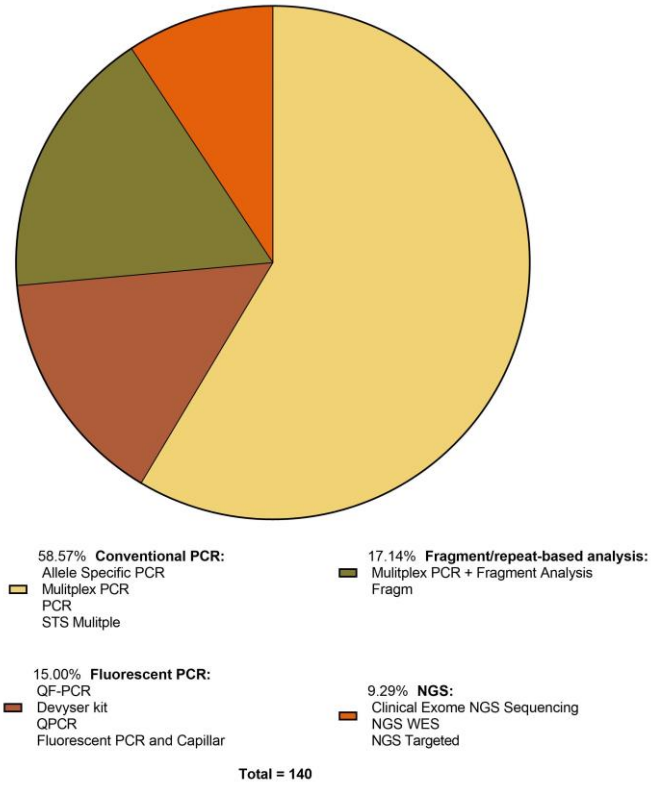
7. Results: Methodology used



8. References

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3. ISO 15189:2012(en), Medical laboratories — Requirements for quality and competence. https://www.iso.org/obp/ui/#iso:std:iso:15189:ed-3:v2:en.

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5. Kamp C, Hirschman P, Voss H, Huellen K, Vogt PH. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Human Molecular Genetics 9(17), 2563–2572 2000 https://doi.org/10.1093/hmg/9.17.2563

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9. Amendments to this summary EQA report

Version	Page	Section	Change	Published
1	-	-	None	11th July 2023
2	5	Appeals	Updated with outcome of appeals	13th Sept 2023
3				

10. Authorisation

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

Dr. Simon Patton on 13th Sept 2023
CEO

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