

2010 CEQA External Quality Assessment for prenatal diagnosis

The CEQA Scheme for prenatal diagnosis provided two amniotic fluid cases. Clinical information, metaphase images and additional technical details were provided on the CEQA website for participating laboratories to analyse and report.

The prenatal diagnosis cases were referred because of ultrasound anomalies (case 1) and a maternal Robertsonian translocation (case 2). Details of rapid aneuploidy screening by QF-PCR and FISH results were available on the website in addition to metaphase images. One case was an abnormal male karyotype with trisomy 13, 47,XY,+13 and the other was a normal female karyotype, 46,XX.

EQA assessment criteria

A panel of cytogeneticists with a wide range of experience undertook assessment of the participants' returns. The assessors considered the following when marking the returns:

- The accuracy of the analysis;
- Whether appropriate cytogenetic tests were undertaken, including any additional studies necessary to make a thorough interpretation of the findings;
- Appropriate interpretation of any single cell anomalies, incomplete cells or technical artefacts;
- The interpretation of the significance of the result in relation to the clinical summary given on the request card;
- The accuracy, clarity and clinical relevance of the report issued to the referring clinician, with reference to the European Cytogenetic Guidelines (www.biologia.uniba.it/eca/).

Assessors

A specialist group of assessors undertook the analysis of the returns, but referred back to the postnatal panel in cases where particular problems required additional scrutiny.

The Panel of Assessors		
Assessor	Location	Role
Martine Doco-Fenzy	Reims	Prenatal assessor
Brigitte Faas	Nijmegen	Prenatal assessor
Daniela Giardino	Milan	Prenatal assessor
Ros Hastings	Oxford	Scheme Co-ordinator, assessor
Karsten Held	Hamburg	Prenatal assessor
Antonio Novelli	Rome	Prenatal assessor
Carmen Ramos	Madrid	Prenatal assessor

Assessors were evaluating the following information (see European Cytogenetic Guidelines for the comprehensive list):-

1. Patient demographic information present
2. Reason for referral
3. Sample type
4. Correct analysis
5. Karyotype given correctly in ISCN 2009
6. FISH ISCN given if appropriate (i.e. where significant information regarding the abnormality depended on FISH)
7. Clear unambiguous written description
8. Syndrome, if appropriate.
9. Consistent with ultrasound findings and/or the referral reason, if appropriate.
10. Assessment of whether there is a risk of recurrence.
11. Whether prenatal diagnosis/testing should be considered in future pregnancies.

12. Onward referral to a Clinical Geneticist, when appropriate – together with an explanation of why the referral is required.
13. Request parental bloods, if applicable - also put into context (i.e. risk of recurrence)
14. Request for appropriate follow up samples (optional) (section 5.2.3 of guidelines).

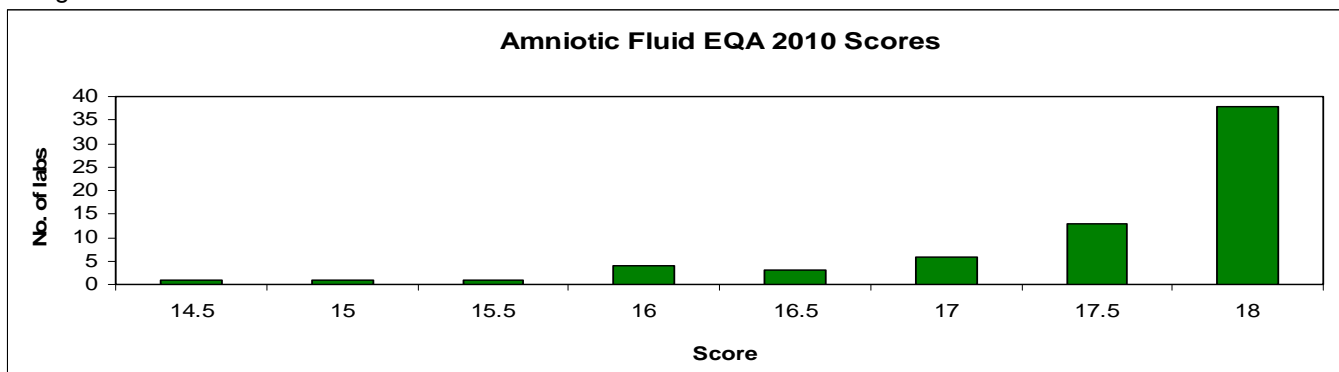
Participation

Prenatal amniotic fluid assessment. Number of Laboratories	
Registered	75
Participated	70
Satisfactory performance	69
% satisfactory	99%

Performance

There was an improvement in the quality of the submitted reports this year.

Only two categories of performance in any single EQA are defined: **satisfactory** and **poor**. Satisfactory performance is defined as the standard that should be achievable by all laboratories. The assessors have discussed at length the possibility of giving individual positive feedback to those laboratories that perform well and score highly, but have concluded that it is difficult to do this fairly and equably. At this point, it should be stressed that the assessors and Steering Committee were pleased to see the majority of participants demonstrating a competent approach to the EQAs, and they would like to congratulate those who achieved high marks in their submissions. Participants are encouraged to refer to the histograms showing the performance of all the laboratories and decide through their own benchmarking whether they consider their performance to be acceptable (maximum score is 18). One laboratory was only marked for Case 1 and their score was not included in the histogram.



Laboratories are encouraged to review their individual reports and contact the CEQA Office if there are any clerical errors. Assessors expend considerable time and effort in making judgments that are as fair as possible and are anxious that those laboratories needing to make improvements will understand that the purpose of the EQA scheme is towards education and the raising of standards. The assessors sincerely hope that participants will respond positively to their comments.

One laboratory received a poor performance designation in this EQA, that was given for an analytical error in Case 2. Poor performance inevitably follows a **critical error**, defined as an error or omission that would have significant disadvantageous clinical consequences for the fetus or family. Any critical error is given a zero score in the relevant category in which the error occurred and in some instances leads to zero or low scores in other categories: for example, it is not possible to give a score for interpretation if the cytogenetic analysis was not correct. This year the Clerical Accuracy was not marked if there was an analytical error.

Case	Critical errors (number of participants)			Total labs participated
	Genotype	Clerical	Interpret	
47,XY,+13	0	0	0	70
46,XX	1	0	0	70

Prenatal EQA

Many laboratories analysed all the metaphases available on the website. While this was reasonable in case 2 if pseudomosaicism was suspected, for case 1 (trisomy 13) fewer metaphases could have been analysed (see European Cytogenetic Guidelines).

Case 1:

All participants succeeded in detecting the trisomy 13. Three laboratories thought there was a paracentric inversion of 1q23q32 in one culture. However, this may have been due to the resolution of bands in 1q31 and not due to pseudomosaicism.

The most common problems were either an incomplete written description, not mentioning Patau syndrome (or Trisomy 13 syndrome), or not relating the karyotype to the ultrasound findings. Where rapid aneuploidy screening was done, a few laboratories did not mention that the two results (QF-PCR/FISH and the cultured amniotic fluid) were concordant.

Most laboratories referred to the recurrence risk of aneuploidy or trisomy 13 in the reports. A recent publication by De Souza et al. (2009) has confirmed the data of Warburton et al. (2004) by comparing the observed number of subsequent trisomies with the expected number of subsequent trisomies based on maternal age-related risk. Women below the age of 35 have a relative risk of 7.8 for another trisomy 13 and a relative risk of 1.6 for a different aneuploidy; while for women over 35 the relative risks are 2.2 and 0.9 respectively. Some laboratories mentioned the possibility of gonadal mosaicism for trisomy 13. The literature shows this is extremely rare as the vast majority cases of free trisomy 13 arise from premature chromatid separation.

Depending on local policy, either the obstetrician or a genetic counsellor will explain the result and the possible consequences to the patient. Consequently, no comment was made on the individual laboratory reports where a referral to a clinical geneticist was omitted.

Case 2:

Several laboratories identified cultural artefacts in incomplete cells as not relevant, three other laboratories considered this artefact to be pseudomosaicism. One laboratory incorrectly concluded that this was a mosaic karyotype, 47,XX,+mar[4]/46,XX[26]. In addition, one laboratory's report was not marked as there was an error on the website that may have caused confusion leading to the wrong conclusion being reached.

The finding of a normal karyotype when the mother is a carrier of a balanced Robertsonian rearrangement does not require UPD studies. Many laboratories suggested maternal and paternal UPD14 should be investigated. There is no increased risk for maternal UPD14, as the only possible mechanism by which UPD14 can occur (with a normal karyotype) is by monosomic rescue resulting in upd(14)pat (see Fig. 1 below). There is only one published case of upd(14)pat in a child with a normal 46,XX or 46,XY karyotype from a maternal der(13;14) Robertsonian translocation carrier (cited in Dawson et al., 2010). However, the risk of upd(14)pat is still extremely low such that UPD testing is not necessary as the studies to date are too small to achieve statistical significance (see ACC Professional Guidelines; Cox et al., 2004; Sung-Ryul & Shaffer, 2002).

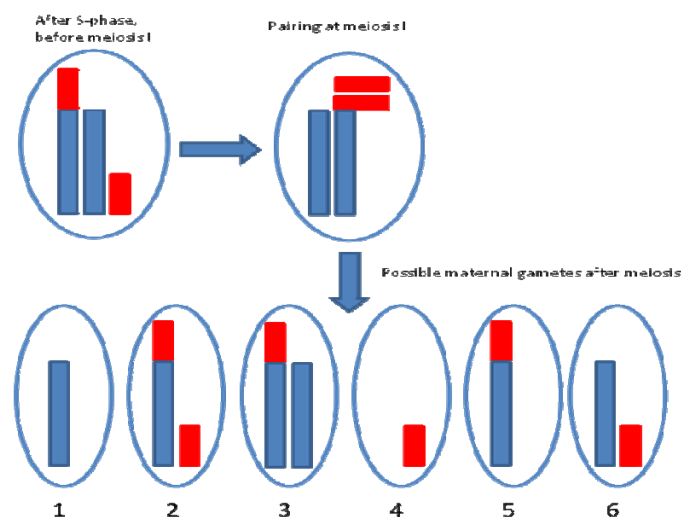


Fig. 1: Behaviour of maternal chromosomes 14 and 21 during meiosis and possible gametes. After fertilization with a normal male complement, gamete 3 can give rise to a upd(14)mat as a result of trisomic rescue and gamete 4 to upd(14)pat as a result of a monosomic rescue (see also Gardner & Sutherland, 2004 pp126-128). **KEY: red - maternal chromosome 21, blue - maternal chromosome 14.**

34/70 laboratories mentioned in their report that the fetus has a normal female karyotype and had not inherited the maternal Robertsonian translocation. Assessors considered this good laboratory practice as it links the result to the referral reason, reassures the reader of the report that the fetus is not a balanced translocation carrier and therefore does not have the same reproductive risk as the mother.

Although this patient is a known translocation carrier it is helpful to reiterate her reproductive risks and that prenatal diagnosis is appropriate in future pregnancies. When mentioning the recurrence risk, it is important not only to mention carriers of the der(14;21) have not only a risk of a trisomy 21 fetus but also miscarriages and the rare possibility of upd(14)mat.

General Recommendations

Many laboratories made errors in ISCN for the aneuploidy FISH. See exemplary letter as well as ISCN 2009 (p113) for the correct ISCN.

In general, assessors considered that some laboratories did not present the prenatal results in a logical pattern in their report. The exemplary reports at the end of this summary letter will hopefully enable laboratories to adopt a more systematic approach to their report writing. The reports should include the patient demographics including the referral reason given in the EQA case (see exemplary report). The patient details were omitted on 25/140 reports. In future EQAs an omission of the patient details will be penalised. For both EQA cases, the mother wanted to know the fetal sex (see referral card). Six laboratories did not give this information in the written description for at least one case.

Participants are urged to refer to the European Cytogenetic Guidelines which gives advice on report content. A report should give details of analytical results and an interpretation of the significance of the result, including an assessment of recurrence as appropriate, and recommendations for clinical or laboratory follow up. Laboratory reports should avoid directive comments such as 'terminate the pregnancy is advised'.

If the cytogenetic laboratory itself does not routinely offer interpretive reports, CEQA requests that the Clinical Geneticist making the final interpretation should be involved in the EQA submission. The purpose of CEQA is to examine the service provided to the patient, not just the technical ability of the laboratory.

Scheme compliance

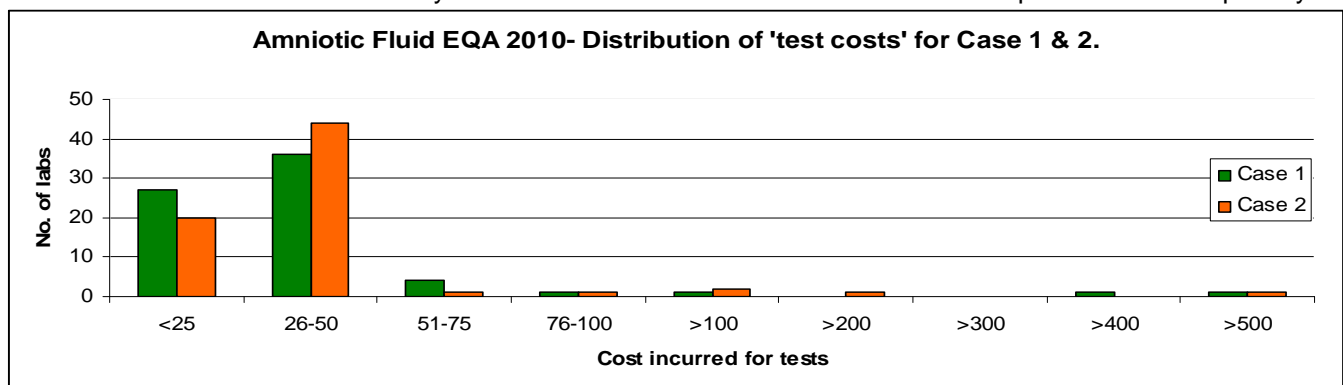
One laboratory did not anonymise their reports.

Participants are encouraged to use the comments section below the report (on the website), for comments to the assessors and also to inform the assessors of any National policies that might affect the content of the report.

Cost of analysis

For cases 1 & 2, it should have been possible to obtain the result with one rapid aneuploidy screening test and G-banding giving a cost of 17 units.

A few laboratories looked at too many tests on the website and laboratories with >150 points incurred a penalty.



References

1. ACC Prenatal Diagnosis Best Practice Guidelines: Prenatal diagnosis www.cytogenetics.org.uk
2. European Cytogenetic Guidelines www.biologia.uniba.it/eca/
3. Cox, H., Bullman, H. and Temple, I. K. (2004) Maternal UPD(14) in the patient with a normal karyotype: clinical report and a systematic search for cases in samples sent for testing for Prader-Willi syndrome. *American Journal of Medical Genetics*, 127A, (1), 21 - 25. (doi: [10.1002/ajmg.a.20611](https://doi.org/10.1002/ajmg.a.20611))
4. Dawson, A.J., Chernos, J., McGowan-Jordan, J., Shetty, S., Steinraths, M., Jia-Chi, W., Xu, J. (2010). CCMG guidelines: prenatal and postnatal diagnostic testing for uniparental disomy. *Clin. Genet.*, 1-7 (doi: [10.1111/j.1399-0004.2010.01547.x](https://doi.org/10.1111/j.1399-0004.2010.01547.x))
5. De Souza E, Halliday J, Chan A, Bower C, Morris JK. 2009. Recurrence risks for trisomies 13, 18, and 21. *Am. J. Med. Genet. Part A* 149A:2716-2722.
6. Sung-Ryul K and Shaffer LG. Robertsonian Translocations: Mechanisms of Formation, Aneuploidy, and Uniparental Disomy and Diagnostic Considerations. *Genetic Testing*. September 2002, 6(3): 163-168
7. Warburton D, Dallaire L, Thangavelu M, Ross L, Levin B, Kline J. 2004. Trisomy recurrence: A reconsideration based on North American data. *Am. J. Hum. Genet.* 75:376-385.

Exemplary reports and assessors comments

Tables of specific comments that were made in each of this year's EQA cases accompany the exemplary reports below

Prenatal Sample, Case 1

Patient demographics:

Susanna JOY; DOB: 19/04/1988.

Date of sampling and receipt: 13/05/10

Tissue: Amniotic Fluid

Sex (Mother): Female

Referral reason: Abnormal ultrasound - holoprosencephaly, proboscis, heart anomaly, ?abnormal kidneys

Karyotype ISCN: 47,XY,+13

With FISH ISCN: 47,XY,+13.nuc ish(DXZ1x1,DYZ3x1,D18Z1x2),(RB1x3,D21S341x2)

Report

Analysis of metaphases from the amniotic fluid sample showed an abnormal male* karyotype of 47 chromosomes including three copies of chromosome 13. The result is concordant with the abnormal FISH (or QF-PCR) results (see report ref xxx/xxx)**.

Trisomy 13 is indicative of Patau syndrome and this result is therefore consistent with the abnormalities observed on ultrasound.

The risk of a future aneuploid pregnancy is increased above the age-related risk; therefore, prenatal diagnosis is advised in any future pregnancy (De Souza et al., 2009). The patient and her partner may wish to be referred for genetic counseling.

Optional: Follow up samples of the fetus/child at delivery should be sent to the Cytogenetics dept.

* fetal sex requested on the referral card.

** nuc ish has been given in the exemplary report karyotype but would not be put in the ISCN if this result had been previously reported.

Points deducted	Prenatal Case 1 Comments
Clerical accuracy	
0.5	Minor FISH ISCN error. See ISCN 2009, page 113.
0.5	The written description is not complete (see exemplary report).
0.5	The patient requested to know the fetal sex.
0.5	The written description does not conform to European Cytogenetic Guidelines- see exemplary report.
See comment in this letter	Patient details should be given on the report.
Genotyping	
0.5	Additional unnecessary tests were undertaken.
Interpretation	
0.5	The report should state there is an increased recurrence risk.
0.5	The report should mention that the ultrasound abnormalities are consistent with the karyotype.
0.5	The report should state there is an increased recurrence risk and prenatal diagnosis is appropriate.
0.5	A recurrence risk can be given without the need for parental blood analysis.
0.5	Patau syndrome or Trisomy 13 syndrome should be mentioned in the report, as there are ultrasound abnormalities compatible with the syndrome (see European Cytogenetic Guidelines).
0.5	The report should mention that the ultrasound abnormalities are consistent with the karyotype.
0.5	Cytogenetic reports should not be directive. It is inappropriate to advise a termination of pregnancy.

Prenatal Sample, Case 2

Patient demographics:

Jane COOMBS. DOB:22/02/1973.

Date of sampling and receipt: 13/05/10

Tissue: Amniotic Fluid

Sex (Mother): Female

Referral indication: Maternal age 37. Mother is a carrier of der(14;21)(q10;q10)

Karyotype ISCN: 46,XX

Report

Analysis of metaphases from the amniotic fluid showed a normal female* karyotype. The fetus therefore does not carry the maternal der(14;21) Robertsonian translocation. This result is consistent with the normal result obtained by QF-PCR (or FISH) (see previous report xx/xxx).

Although monosomy rescue resulting in paternal upd14 is unlikely (ACC guidelines, Dawson et al., 2010), uniparental disomy testing of chromosome 14 was investigated and showed normal biparental inheritance**.

As the mother is a carrier of a der(14;21) translocation, prenatal diagnosis should be offered in future pregnancies***.

Optional: Follow up samples at delivery should be sent to the Cytogenetics dept.

* fetal sex requested on referral card

** this is optional due to the extremely rare possibility of UPD14 with a normal karyotype. Most reported cases of UPD14 have had been associated with a Robertsonian translocation. There is only one reported case of UPD14 in a normal karyotype (Dawson et al., 2010).

***Optional: Patient has already been seen by a Clinical Geneticist but reports should 'stand-alone' and therefore it is helpful to reiterate this information.

Points deducted	Prenatal Case 2 Comments
Clerical accuracy	
0.5	Minor FISH ISCN error. See ISCN 2009, page 113.
0.5	The report should state this is a normal female karyotype.
0.5	The patient requested to know the fetal sex.
0.5	Unnecessary tests undertaken.
Genotyping	
3.0	Critical analytical error:
Interpretation	
3.0	Cytogenetic reports must include an interpretation of the results – see exemplary report and European Cytogenetic guidelines (Section 8).
3.0	Analytical error, therefore interpretation incorrect.
0.5	As the fetus has a normal karyotype, the only UPD that could arise is paternal UPD14.
0.5	As the fetus has a normal karyotype, the mechanism by which maternal UPD14 would arise is highly unlikely (see summary letter).
0.5	Paternal and maternal UPD will have been excluded from the UPD studies. As the fetus has a normal karyotype, the only UPD that could arise is paternal UPD14.