Expert Review 03

QF-PCR and maternal cell contamination

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About the author

Kathy Mann, PhD, is a Principal Clinical Scientist within the Cytogenetics Department, GSTS Pathology, based at Guy's Hospital, London UK. With colleagues she developed and established the first QF-PCR service for the rapid detection of prenatal aneuploidy in the UK National Health Service (NHS) in 2000, a service which now processes >4,000 samples/year. The laboratory has since published more than 15 peer-reviewed papers related to the field of QF-PCR including new service developments such as stand-alone testing, which have subsequently been adopted by other laboratories, and their recent report detailing the results of the more than 40,000 prenatal samples. Other publications detail QF-PCR assays and best practice and describe phenomena such as mosaicism, maternal cell contamination, CNV and somatic microsatellite mutations. Kathy co-authored the first draft of the UK QF-PCR Best Practice Guidelines (2005) and has edited subsequent versions including the 2012 Guidelines. She has been an assessor for the Molecular Rapid Aneuploidy (MRA) UK NEQAS since its inception in 2004 and she was a speaker and joint organiser of the International QF-PCR Workshops from 2003-2008.

Introduction

Prenatal diagnosis currently involves testing material that is obtained by chorionic villus (CV) or amniocentesis sampling; either of these procedures may result in contamination of the sample with maternal cells and the potential for misdiagnosis. It is important to identify the presence and level of maternal cell contamination (MCC) in a prenatal sample as this may have implications for sample processing and result interpretation.

The removal of maternal decidua from chorionic villi (CV) samples is standard practice prior to testing and therefore MCC is rarely observed in CV samples of usual quality. However, for CV samples of poorer quality, removal of maternal material may be technically difficult; such samples may retain maternal material and give rise to MCC of uncultured and/or cultured villi.

MCC of amniotic fluid (AF) occurs more frequently and is usually due to the presence of maternal blood cells, indicated by blood-staining of the AF, although it should not be assumed that all blood is maternal in origin. The presence of maternal lymphocytes in uncultured AF can cause significant problems, but is rarely an issue for cultured amniocytes as maternal lymphocytes are lost during the culture process. If MCC is detected in uncultured AF in the absence of blood-staining, or in cultured amniocytes, it is likely to be due to the presence of small fragments of maternal tissue removed by the sampling needle during the amniocentesis. It is important to note that cells from maternal tissue fragments may proliferate during the culture process.

Detection of MCC by QF-PCR

The principle and application of QF-PCR analysis for the detection of aneuploidy in prenatal samples is described in Expert Review 01: An introduction to QF-PCR; the strategy uses short tandem repeats (STRs) to determine sequence copy number and also provides a genotype of the tested sample. This has the benefit of bringing to light additional genotypes which may represent MCC, mosaicism (discussed in Expert Review 02: Detecting mosaicism with QF-PCR), chimerism, a twin pregnancy or external contamination. MCC gives characteristic allele patterns of skewed and normal biallelic ratios and triallelic results in the form of allele A + allele B = allele C where alleles A and B have the smallest peak area/height and are specific to either the mother or the fetus whilst the largest allele C is the shared fetal-maternal allele (Figures 1 and 2). These allele patterns are observed for STRs on every chromosome and can be distinguished from mosaicism in most cases. Biallelic results with a normal 1:1 ratio are observed when the maternal genotype is the same as the normal fetal genotype.

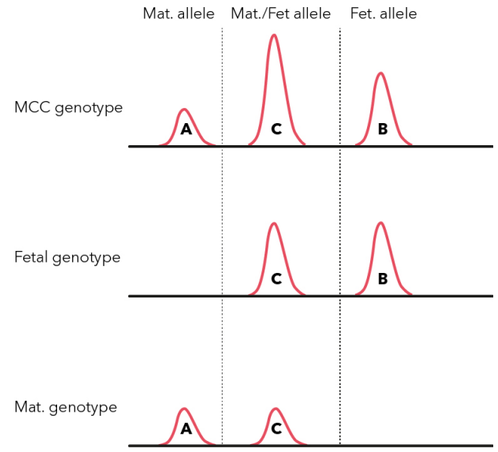

Fig.1 Illustration showing a typical triallelic MCC genotype (top) where A+B=C. The maternal genotype (alleles A and C) and fetal genotype (alleles B and C) are also given.

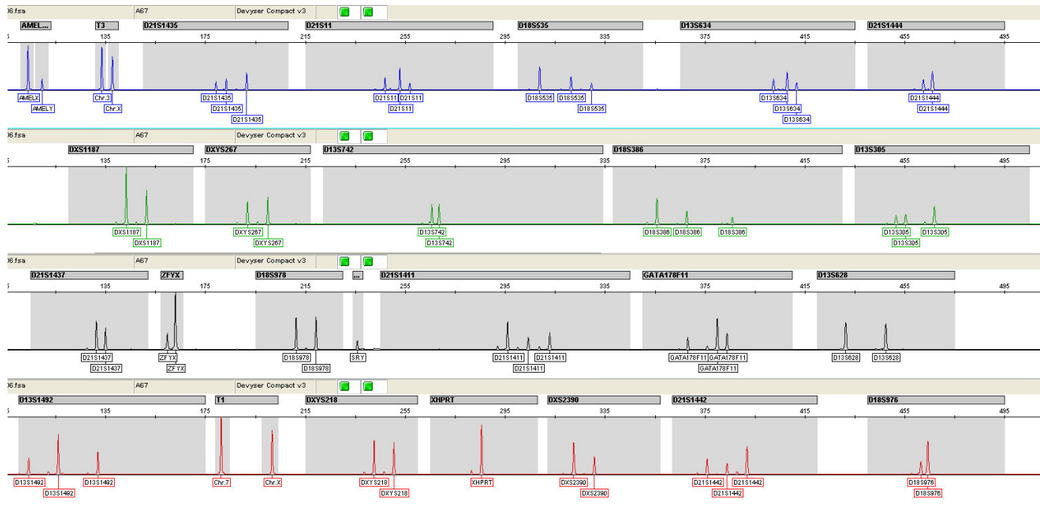

Fig. 2 QF-PCR trace showing a sample with significant MCC (one genotype contributes approximately 35% and the other 65%). Although the maternal and fetal genotypes are not identified, all ten of the triallelic STRs show the A+B=C pattern consistent with MCC. STRs on every chromosome are affected and there are no STRs showing four alleles.

The absence of four-allele results indicates that the second genotype is very unlikely to be due to chimerism, a vanished twin or DNA from an external source. The level of the contaminating maternal genotype will vary and may be the minority or majority genotype. Either way, all triallelic results should exhibit the A+B=C allele pattern.

Incidence of MCC

Stojilkovic-Mikic et al., 2005, used QF-PCR analysis to investigate maternal cell contamination in uncultured and cultured CV (53) and AF (254) samples. None of the CV samples were found to have MCC. 9% of uncultured AF samples exhibited a second genotype, consistent with MCC, with the majority of these (82%) showing some level of blood-staining. 3% of the AF samples exhibited MCC in a single culture, but this did not correlate with the presence of blood-staining and was therefore consistent with maternal contribution from a tissue plug. It is important to note that blood-staining was only visible in 1% of AF samples prior to centrifugation and that in the majority of cases blood-staining was only apparent after centrifugation and inspection of the pellet. The level of blood-staining in the pellet did indeed correlate with the likelihood of MCC in the uncultured AF (Figure 3). In most cases, the second genotype was present at a low level and the majority genotype could be interpreted and reported. However in two cases (<1% of the AFs) the maternal genotype was too high to allow safe interpretation of the fetal genotype and therefore no rapid QF-PCR result was available. In larger data sets, the incidence of AF samples classed as unsuitable due to a significant second genotype (assumed to be maternal) is higher and in our latest cohort is 2.24% (Mann, 2012). In addition, 0.25% CV samples are classed as unsuitable because of the presence of a second genotype, consistent with MCC. A further 0.78% CV samples exhibit low levels of MCC.

"in the majority of cases blood-staining was only apparent after centrifugation and inspection of the pellet"

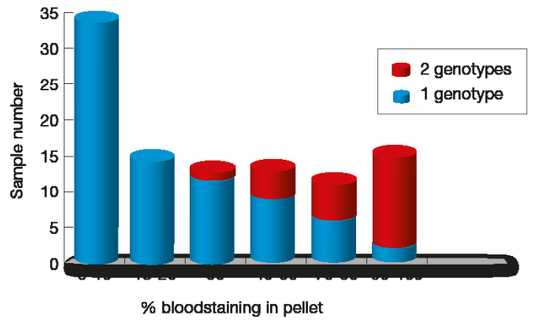

Fig.3 Number of blood-stained AF samples plotted against the degree of blood-staining in the cell pellet following centrifugation. Blue columns show samples with a single genotype following QF-PCR testing of uncultured material whilst Red columns show samples with two genotypes (Stojilkovic-Mikic, 2005).

Result interpretation

In the case of CV and AF samples, if two genotypes are present at a level that results in one or more inconclusive (0.65-0.8 or 1.5-1.8) STRs then the majority genotype should not be interpreted, in line with the UK Best Practice Guidelines, 2012. This level of MCC may complicate the interpretation of the fetal genotype. The sample is therefore categorised and reported as unsuitable for QF-PCR analysis and the pattern of the two genotypes as being consistent with MCC. All such samples can be cultured and analysed using QF-PCR, karyotype analysis or aCGH, depending on the referral indication. If two genotypes are detected in a blood-stained AF sample, then it is very likely that the cultured cells will exhibit a single fetal genotype as maternal lymphocytes are lost during the culturing process. However, for clear AF samples and CV samples that exhibit two female genotypes, the results from both the uncultured and cultured cells should be interpreted with care, as the majority cell line may be maternal in origin; a maternal blood genotype may be required for confident result interpretation. In our laboratory, a result from cultured cells is not possible in 3% of MCC cases (Mann, 2012) because of insufficient fetal cells being present in the sample.

Heavily blood-stained AF samples

In rare cases, a single or majority genotype may represent the maternal genotype. Such cases are usually indicated by high levels of blood-staining in AF. Therefore, in cases where the cell pellet prepared for QF-PCR analysis appears to be predominantly blood-stained (we categorise these samples by the absence of a visible white cellular layer above the red cell pellet) it is recommended that the result is interpreted with caution. In our laboratory, all heavily blood-stained AF samples are sexed and those that have a single or majority male genotype are reported as usual. For heavily blood-stained samples that have a single female or mixed female genotype with no inconclusive allele ratios, we request a maternal blood sample in order to ascertain the origin of the reportable genotype. In our laboratory, 0.54% of AF samples are heavily blood-stained and exhibit a single or predominant female genotype (Mann, 2012). Of these samples, for which a maternal blood sample is received, 83% of the genotypes are found to be solely or predominantly fetal in origin and a result is therefore available.

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Summary

The ability to identify MCC in uncultured AF and CV samples minimises the risk of misdiagnosis due to the analysis of maternal cells. Thus, a QF-PCR based approach offers a real benefit over both Fluorescence in Situ Hybridisation (FISH) analysis of interphase nuclei (Benn and Hsu, 1983) and other molecular methods of rapid aneuploidy diagnosis such as MLPA (Gerdes, 2008) and BACs-on-Beads (Vialard, 2011), where MCC of samples from female pregnancies cannot be detected; heavily blood-stained AFs are not usually analysed using these techniques. In contrast, all prenatal samples regardless of quality can be tested using a QF-PCR strategy; poor quality CV samples and heavily blood-stained AF samples where material is of uncertain origin can be genotyped and compared to the maternal genotype. For the majority of poor quality samples, this strategy gives a confident and reportable result.

References

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