

Practice guidelines for the Testing for maternal cell contamination (MCC) in prenatal samples for molecular studies.

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1. INTRODUCTION

The potential presence of maternal cell contamination (MCC) in chorionic villus (CVS) or amniotic fluid (AF) samples poses a serious risk for prenatal misdiagnosis. This risk is of particular concern where samples are to undergo molecular prenatal testing as assays are largely PCR-based. The magnitude of the risk of MCC depends on variables such as sampling technique, sample quality, operator and method of sample processing.

CVS usually contain maternal decidua which is removed through careful dissection by the local Cytogenetics laboratory. Maternal cell populations may be increased in culture and therefore both uncultured and cultured CVS may be at risk of MCC.

In AF samples, bloodstaining is a major indicator of the possibility of MCC (although the blood can also be fetal in origin). Uncultured amniotic fluid has a higher frequency of MCC than cultured amniocytes, as growth of the amniocytes is enhanced during the culturing process.

Investigations to exclude MCC are currently addressed by laboratories individually on a case-by-case basis, however, there is a need for standardisation of the approach to ensure optimal and accurate prenatal patient care.

A CMGS best practice meeting was held on the 29th June 2007, at Birmingham Women's Hospital to discuss and formulate best practice guidelines for MCC testing, with respect to prenatal samples that are to undergo further molecular testing. This meeting included representatives from 30 CMGS labs and also observers from the ACC. Prior to the meeting, a questionnaire was sent to all participating labs and the collated data was discussed with a view to assessing current diagnostic practices and agreeing minimal standards for best practice. These guidelines were drawn up following discussion and agreement of the points at the Best Practice Meeting.

Each guideline is described as either:

- **Recommended** practice which should be implemented to ensure quality of service
- **Acceptable** practice, where more than one approach is satisfactory
- **Not acceptable**, which highlights areas where the quality of service may be compromised.

The testing of prenatal samples is complicated by limited sample quantity, variable sample quality, mosaicism and maternal cell contamination. The processing of a number of prenatal samples requires stringent quality control measures to minimize the risk of sample mix-up. These aspects of prenatal diagnosis are taken into account in the following guidelines.

2. SERVICE OVERVIEW

2.1 General Recommendations

It is **recommended** that all prenatal diagnoses for single gene disorders should have MCC studies carried out to exclude as far as possible the presence of MCC which might compromise the validity of the molecular prenatal result. 'MCC studies' encompasses a number of approaches which are discussed below. It is **recommended** that the local Cytogenetics laboratory establishes CVS or amniocyte cultures whenever possible to act as a back up for testing, in case of insufficient sample or MCC in the uncultured sample.

It is **recommended** that the lab performing the molecular prenatal test should also carry out the MCC testing, however it is recognised that due to financial constraints this is not always possible. Testing by a single lab ensures that the minimum level of MCC that may affect the molecular prenatal test may be directly compared with the sensitivity of the MCC assay (see below for further information). It also ensures that the molecular prenatal report is not issued before MCC testing is complete, which would potentially compromise the prenatal result, and that all information is available to the testing laboratory. Where the prenatal molecular testing is not

performed in an accredited laboratory (CPA or equivalent) it is **recommended** that MCC testing is performed by the laboratory exporting the sample. A significant level of MCC is defined as equal to, or greater than, the minimum level of MCC that has been shown to affect the results of the specific prenatal molecular assay (see later section entitled Assay Sensitivity). This expression is used throughout the guidelines.

If sexing of a prenatal sample is required prior to possible export it is **acceptable** that this is carried out by the exporting laboratory. Sexing of a prenatal sample as male is not sufficient to exclude MCC.

2.2 Sample type

It is **recommended** that MCC should be assessed in any DNA sample used for prenatal molecular testing. The MCC check should always be performed on the same DNA sample used for prenatal testing. It is **acceptable** to perform prenatal testing and MCC checks on DNA from the following

- Uncultured CVS (including bulk CVS preparations and CVS fronds),
- Cultured CVS
- Amniotic fluid samples.(cultured or uncultured)

It is **recommended** that MCC is assessed in fetal blood samples and cord blood samples.

It is **recommended** that MCC is assessed in products of conception (POC) unless the molecular assay provides confirmation of the prenatal test result, and is informative regarding absence of detectable MCC.

2.3 Parental samples

It is **recommended** that analysis of a maternal sample is carried out alongside the prenatal sample. It is **not acceptable** to state that significant MCC has been excluded if a maternal sample has not been analysed. For example it is not acceptable to rely on the absence of additional microsatellite alleles in a prenatal sample in a QF-PCR based aneuploidy assay.

If a maternal sample is not available then the fact that MCC has not been excluded should be stated on the report.

It is recognised that under some circumstances (for example prenatal diagnosis by linkage analysis, haemoglobinopathies) it is good practice to analyse a paternal sample in addition to the maternal sample.

However, in the absence of such clinical reasons, it is **recommended** that analysis of a paternal sample should be avoided as this may raise non-paternity issues. If a test that determines paternity is carried out, then the referring clinician should be made aware that the test may reveal non paternity.

2.4 Assay type

The choice of assay used to assess MCC is down to local practice. Thirteen out of 26 labs that replied to the questionnaire use commercial ID kits such as Powerplex (Promega), AmpFlstr (Applied Biosystems), Profiler (Roche), Human ID kit (Beckman) to exclude MCC.

Nine labs currently use their QF-PCR based rapid aneuploidy assay. The remainder use “in-house” marker mixes.

It is **recommended** that where the prenatal test does not provide evidence to exclude significant MCC, that evidence from a minimum of 2 microsatellite markers is required to report that significant MCC has been excluded. (Note: that where the non inherited maternal allele coincides with a stutter peak of a fetal allele this marker should not be used as an informative marker to exclude MCC, as low level MCC may be masked). Where evidence from the prenatal test result is considered sufficient to exclude the possibility of MCC, then this should be subject to the same sensitivity criteria as for the MCC assay (see Assay sensitivity), and that testing of a maternal sample has also been carried out to exclude the possibility of SNPs affecting the assay.

It is **acceptable** to include chromosome 21, 13, 18, X and Y markers in the MCC assay provided the patient has consented to QF-PCR or karyotype analysis. If this is not the case, then it is **recommended** that there is prior discussion with the referring clinician / referring laboratory before using these markers in the MCC assay. If the patient specifically requests that chromosome analysis is not carried out it is **recommended** that markers located on chromosomes 13, 18 and 21 (involved in the common trisomies), and the sex chromosomes are not used for MCC testing. It is recognised that anomalies can be detected with markers from any of the chromosomes, however avoidance of markers located on chromosomes 13, 18, 21, X and Y in the assay will minimise the risk of clinically significant chromosome abnormalities being detected.

2.5 Assay sensitivity

It is **recommended** that laboratories should establish the sensitivity of their chosen MCC assays using carefully designed DNA mixing experiments, for example replicating MCC by mixing two parent / child DNA samples in varying proportions - 1%, 2%, 5%, 10%, 20%, 50% & 100%. It is **recommended** that the chosen MCC assay should routinely be capable of detecting at least a 10% level of MCC. The sensitivity should be assessed regularly to determine any batch-to-batch variation of the kit/assay in use. It is **recommended** that the sensitivity of the MCC assay is also defined with regard to minimum and maximum total input DNA/DNA concentrations, as weak genotype data may reduce the sensitivity of the MCC assay. For fluorescent analysis, this can be based on peak height data and should be in accordance with the genetic analyser’s specification and/or based on a smooth baseline.

Laboratories should be aware that the sensitivity of their specific molecular prenatal assays may differ from the sensitivity of the MCC assay. Ideally the sensitivity of the MCC assay should be equal to or greater than the specific molecular prenatal assay.

However this is not always possible as certain specific assays are known to be very sensitive to MCC (e.g. deletion & TP-PCR based tests). It is **recommended** that laboratories should determine the sensitivity of all their relevant assay types using the same methods as for the MCC assay.

Where the molecular prenatal test is more sensitive to contamination than the MCC assay it is **recommended** that an alternative test such as Quantification/Southern blotting/disease-linked markers is carried out to confirm the prenatal genotype result.

2.6 Reporting

It is **recommended** that the wording on the molecular prenatal report should be “Significant maternal cell contamination of the

fetal DNA sample has been excluded” as it is not possible to exclude very low level MCC. Where the MCC testing has not been done by molecular prenatal testing laboratory and has been carried out by the exporting laboratory (see “Service Overview”), it is **recommended** that the sensitivity and method of MCC analysis is included on the MCC report issued by the exporting laboratory. It is **recommended** that this report is copied to both the referring clinician and the molecular prenatal testing laboratory. It is **recommended** that the laboratory reporting the molecular prenatal test states which laboratory has performed the MCC testing on their report. It is **not acceptable** for the molecular prenatal report to be issued before MCC testing is complete.

Where MCC is detected at low levels it does not necessarily invalidate the prenatal test result. The prenatal test result and sensitivity should be considered alongside the MCC assay result and sensitivity, and a reasoned (but cautious) conclusion should be reached. This will be judged on a case by case basis, for example, detection of a low level of MCC may explain the presence of a faint allele signal (corresponding to the non-transmitted maternal allele) in the PND assay. In some cases it will be necessary to carry out further analysis of cultured cells and/or additional samples before reporting results.

2.7 Reporting copy number variation (CNV)

Occasionally anomalous marker results may be detected in prenatal or parental samples using MCC assays. These may fall into two categories:

1. Potential sub microscopic duplication/deletion (SMD) detected in a prenatal sample. This is covered in the rapid aneuploidy Best Practice Guidelines (see http://cmgsweb.shared.hosting.zen.co.uk/BPGs/best_practice_guidelines.htm).
2. Anomaly detected in parental sample. It is **recommended** that reporting of these results is decided on a case by case basis and is based on local laboratory practice, in consultation with the Clinical Genetics service.

2.8 Reporting times

The majority of labs surveyed initiated MCC testing immediately on receipt of the sample, alongside the prenatal test. It should be noted that under White Paper reporting time guidelines, prenatal tests not involving Southern blotting are subject to a target 3 working days reporting time, where prior warning of testing has been received.

3.0 REFERENCES

Schrijver I, Cherny S.C. and Zehnder J.L. 2007. Testing for maternal cell contamination in prenatal samples. *J. Mol. Diagnostics* **9** (3): 394-400.