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Special Article

Laboratory Guidelines for Detection, Interpretation, and Reporting of Maternal Cell Contamination in Prenatal Analyses

A Report of the Association for Molecular Pathology

Narasimhan Nagan,*† Nicole E. Faulkner,*† Christine Curtis,*‡ and Iris Schrijver*§

From the MCC Guidelines Working Group of the Association for Molecular Pathology (AMP) Clinical Practice Committee; the Molecular Diagnostics Laboratory,† Genzyme Corporation, Westborough, Massachusetts; the Center for Human Genetics Laboratory,‡ University Hospitals-Case Medical Center, Case Western Reserve University, Cleveland, Ohio; and the Departments of Pathology and Pediatrics,§ Stanford University School of Medicine, Stanford, California*

This document summarizes laboratory guidelines for the detection, interpretation, and reporting of maternal cell contamination in prenatal analyses. ***(J Mol Diagn 2011, 13:7–11; DOI: 10.1016/j.jmoldx.2010.11.013)***

Interpretation of prenatal analyses is one of the most complex areas in genetic testing. Clinicians use invasive methods that may increase the risk of pregnancy loss, to obtain chorionic villus samplings (CVS) or amniotic fluid (AF) for prenatal molecular, cytogenetic, or biochemical analyses. Though these samples may be small and contaminated with maternal tissue or hematopoietic cells, accurate and timely test results are imperative given the ramifications associated with these analyses. To provide a correct result interpretation, the laboratorian must be confident that the sample used for analyses is of purely fetal origin. In addition to prenatal diagnosis of inherited molecular, cytogenetic, or metabolic disorders, maternal cell contamination (MCC) analysis may also be used in conjunction with zygosity analysis to rule out the presence of contaminating maternal or co-fetal material in multiple gestation pregnancies. Additionally, MCC analysis can serve as an internal quality assurance measure to ensure that the biological mother is matched with her concordant fetus(es), thereby minimizing the possibility of sample mix-up within a prenatal setting.

Contamination of a fetal or cord blood specimen by maternal cells is a potential source of error in diagnostic prenatal testing. Although contaminating maternal blood can be visualized in 1% to 2% of amniotic fluid samples and in up to 38% of pelleted amniocytes following centrifugation,[1] the origin of this blood—fetal or maternal—cannot be reliably assessed. Even low levels of contamination that are below visual detection may negatively impact molecular, biochemical, or cytogenetic results. Highly sensitive molecular testing methods have identified the presence of MCC in

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Standard of practice is not being defined by this article, and there may be alternatives.

Corresponding author: Iris Schrijver, M.D., Director, Molecular Pathology Laboratory, Stanford University Medical Center, Lucile Packard Children's Hospital, Associate Professor of Pathology and (by courtesy) Pediatrics, Stanford University School of Medicine Pathology Department, 300 Pasteur Dr., Room L235, Stanford, CA 94305-5627. E-mail: ischrijver@stanfordmed.org.

Address reprint requests to The Association for Molecular Pathology, c/o Mary Williams, 9650 Rockville Pike, Bethesda, MD 20814-3993, E-mail: mwilliams@asip.org.

9.1% of direct or cultured fetal cell preparations, 17.8% of which had no visible evidence of maternal blood.[2] MCC occurs at a significantly lower rate among AF cultures than in direct (uncultured) AF samples, because culture conditions favor the growth of amniocytes and reduce or eliminate maternal blood cells.[3] CVS analysis presumes that the fetal karyotype/genotype is reflected accurately in the extraembryonic tissues. Contamination of a CVS sample with cells of maternal origin may result in analysis of the maternal rather than the fetal karyotype or genotype, especially when the sample size is small. In CVS and abortus samples, the culturing process increases the risk of detectable MCC given the colocalization of maternal and fetal cell lineages in the placenta. In these situations, MCC can be reduced by carefully separating the maternal decidua from chorionic villi before setting up the culture for analysis.[4]

Professional organizations such as the American College of Medical Genetics (*http://www.acmg.net/Pages/ACMG_Activities/stds-2002/g.htm*, last accessed August 15, 2010), the Clinical Laboratory Standards Institute (2006 edition of *Standards and Guidelines for Clinical Genetics Laboratories*, prenatal testing section G19 first added in 2003; *Molecular Diagnostic Methods for Genetic Diseases, Approved Guideline—Second Edition*, MM1-A2 Vol 26 No 27), and the Clinical Molecular Genetics Society (CMGS e-publication 2007: *http://www.cmgs.org/BPGs/pdfs%0current%20bpgs/MCC_08.pdf*, last accessed August 15, 2010) in the UK have developed standards and guidelines for cytogenetic and molecular genetic testing that recommend MCC testing in prenatal diagnosis. However, a survey performed in 2007 of prenatal diagnostic practices among 35 laboratories in the US took an evidence-based approach to illustrate a lack of standardization for MCC testing. Only approximately 60% of the surveyed laboratories performed MCC testing on all prenatal samples. There was considerable variability in the utilization and interpretive criteria for MCC analysis.[5] This report included a comprehensive review of literature relevant to MCC testing practices and concluded that there is a need for MCC laboratory guidelines to ensure more standardized, reliable, and accurate results interpretation in prenatal genetic analyses, for optimal quality assurance within the setting of prenatal diagnosis.

This document provides guidelines to use, interpret, and report MCC analysis performed within the clinical laboratory. These guidelines address the entire testing process within a clinical laboratory, from preanalytical, through the analytical (to include technical and interpretative) and postanalytical (reporting) phases of analysis. Together with good laboratory practices and existing standards and guidelines for molecular genetic testing of heritable diseases, these guidelines can help provide accurate genetic information and minimize the potential of diagnostic error.

Preanalytical Guidelines

1. To determine the pure fetal origin of all prenatal specimens undergoing genetic analysis, it is recommended that MCC analysis be performed in parallel with diagnostic testing, regardless of the genetic disorder or its mode of inheritance.

2. The need for a maternal specimen (typically blood or buccal) for concurrent MCC analysis should be communicated to the treating clinicians by the laboratory before prenatal specimen collection.

3. All pertinent intake information including clinical, family, and testing history, should be provided to the testing laboratory, along with the prenatal sample.

4. Appropriate disclosure of biological relationships and representation of maternal parentage are required (for example: a gamete donor or surrogate pregnancy).

5. Information regarding multiple gestations should be available to the laboratory, along with the prenatal sample, due to the risk of potential co-fetal contamination from a twin.

6. Only a maternal peripheral blood or buccal sample is strictly required for MCC testing. Paternal samples are not helpful for MCC analysis.

7. Acceptable specimen types for prenatal analysis include directly obtained, uncultured AF and CVS, cultured AF and CVS, cord blood, peripheral umbilical blood specimens, and products of conception.

8. The median quantity of material requested for direct AF testing is 12 ml and 5 to 10 mg of cleaned, isolated CVS tissue.[5] However, due to the precious nature of these prenatal specimens, testing on samples with volumes below the minimum requested amount may be attempted depending on the policies and practices of the testing laboratory. In such instances, if the results following prenatal analysis are either inconclusive or unclear, it is strongly recommended to confirm the test results on a backup cultured specimen. A fully adherent monolayer of cultured AF or CVS cells at a confluency of at least 75% is preferred for testing. Cultured cells at low confluency or cultures containing nonadherent, dislodged nonviable cells should not be appropriate for testing.

9. Specific prenatal specimen information to be recorded by the testing laboratory should include: the sample type, date of collection, collection container types, sample condition on arrival, tube label check, color of the cell culture media, degree of confluency, and the presence and extent of blood and/or decidua in uncultured amniotic fluid or CVS specimens. Laboratories should have procedures to ensure that every CVS specimen is thoroughly examined under a dissecting microscope to remove any contaminating maternal decidua before setting up both direct as well as long-term CVS cultures. Coordination between the testing laboratory and the cytogenetics laboratory may be needed to ensure this process.

10. Specimen requirements depend on the testing method used. For instance, a smaller sample amount is required for a PCR-based assay versus Southern blot analysis. Also, the sample size obtained may be proportional to gestational age. In general, the risk of MCC is greater early in gestation, because of the relative paucity of fetal cells compared to maternal cells. Robust extraction methods must be used to ensure adequate yield of DNA from prenatal specimens.

11. Backup cultures should be readily available for repeat or confirmatory testing if MCC is detected in an uncultured specimen. The backup cultures must remain available until the testing is completed and results are reported to the ordering physician. Reduced turnaround time and decreased cost of analysis are advantages to testing direct AF specimens; however, there is a greater possibility of contamination with maternal hematopoietic cells. Cultured AF cells, on the other hand, are much more likely to represent a homogeneous specimen type reflecting pure fetal origin due to an absence of coexisting contamination with cells of maternal hematopoietic origin. Maternal blood cells senesce during culture and are not expected to proliferate.[6] Although prolonged culture may allow overgrowth of maternal fibroblasts and epithelial cells, potentially leading to detectable MCC, discarding the first draw of amniotic fluid samples can reduce this possibility.[7]

12. Every effort should be made to include the prenatal sample(s) in the earliest available assay setup and to prioritize the prenatal samples into testing if clinically warranted.

13. It is important that only one prenatal test sample be handled at any one time to ensure accuracy and prevent a potential mix-up of samples at this stage of analysis.

14. The laboratory performing the diagnostic prenatal analysis should preferably also perform the MCC testing. This approach allows the laboratory to provide a comprehensive and centralized result interpretation. This in turn helps to improve turnaround time and ensures quality assurance of the entire process in one laboratory.

Technical Guidelines

1. MCC testing should be performed on DNA extracted from the same sample or subsample, culture or subculture, that was used for concurrent clinical diagnostic testing.

2. Maternal and prenatal specimens should be tested and analyzed for MCC concurrently within the same analysis to allow for a direct comparison of results.

3. A variety of commercial DNA genotyping assays are available for the purpose of identity testing. The performance characteristics of these assays must be validated for the intended purpose of MCC studies in each laboratory before implementation into clinical testing. The basic premise of these assays is the comparison of highly polymorphic short tandem repeat/microsatellite loci between the maternal and fetal DNA samples following PCR and size separation, to assess the risk of MCC in the fetal sample.

4. Tetranucleotide/pentanucleotide markers are preferable monitors of MCC over smaller (eg, dinucleotide) repeat markers due to the superior fidelity, robustness of PCR amplification, accurate measurement of repeat units by fragment analysis, distinguishable alleles with a high discriminative capacity, and intergenerational stability among individuals in the general population.

5. The MCC analysis should use a sufficient number of markers to accurately rule out MCC at the level of sensitivity previously determined by the laboratory during its initial MCC assay validation process. In general, MCC assays that include a larger number of polymorphic markers are preferable because of the increased likelihood of having included multiple informative markers (that clearly demonstrate an unshared allele between mother and fetus) and because markers can differ in their informativeness when the level of MCC is at or near the assay's limit of detection. Using only a couple of markers may provide a false sense of security around apparently absent MCC.

6. If using capillary electrophoresis technology, we recommend that peak height and signal-to-noise ratio thresholds be monitored within the testing laboratory to reliably assess percentage MCC detectable following extraction and molecular amplification. Variation in assay performance, attributable to factors such as input DNA concentrations and analyzer specifications resulting in weak genotype data (such as low peak heights), may affect MCC assay sensitivity.

7. PCR stutter peaks (caused by replication slippage during PCR amplification) that are the size of the repeat unit of a maternal allele should be considered noninformative for MCC studies because low-level MCC may be obscured in this setting. A flat baseline on the fetal sample electropherogram in the region of interest enables the reviewer to detect even a small peak of maternal origin.

8. Identical allelic markers between mother and fetus are uninformative for MCC. It is recommended that two to three informative microsatellite markers reflecting clearly definable, separate maternal and fetal genotypes from among a panel of approximately 7 to 10 markers be used to assess the presence of MCC in a prenatal sample.

9. By using the peak heights or area under the curve for each primary allele peak, the proportion of a contaminating maternal allele to a noncontaminating, nonshared fetal allele can be estimated for each informative marker. The calculated percentage across all informative markers would be expected to approximately correlate with the extent of maternal contamination within a prenatal sample.

10. If supplementary evidence from the clinical prenatal test is taken into consideration to rule out MCC, it is imperative that both the maternal and fetal specimens be tested in the same molecular assay. This allows a direct comparison of results, thereby excluding the possibility of allele dropout attributed to sequence variants and/or polymorphisms that might affect the analysis and the resulting interpretation. For example, if the fetus is negative for the maternal mutation in the diagnostic test, then this finding could be considered as one informative marker for the purpose of ruling out MCC, as long as both the maternal and fetal specimens are tested by the same diagnostic assay methodology.

11. Ideally, the testing laboratory would know the validated sensitivity of each prenatal analysis (based on methodology) to varying amounts of MCC and its effect on result interpretation. A validated MCC assay that is more sensitive than the clinical diagnostic assay will help ensure that low levels of contamination not detectable by an MCC assay do not affect the clinical test result interpretation. Performing both the diagnostic prenatal analysis and MCC testing within the same laboratory allows for

a direct comparison of both results within the context of the sensitivity of each assay.

12. At least 5% MCC must be routinely detectable by the clinical laboratory. This percentage is recommended as the upper limit based on the fact that erythrocyte admixture of just a few percent is readily visible by eye, and that incorrect interpretation of a PCR-based diagnostic prenatal test has been reported even at a level of 1% to 2% MCC,[6] with increasing likelihood at higher percentages.

Interpretive Guidelines

1. Inheritance of a marker presenting as a null allele due to allele dropout and/or a deletion within a marker may cause an apparent discordant result between the mother and the fetus at any particular locus.[8] Somatic changes in allele repeat units of the fetus due to *de novo* or post-zygotic mutations are another reason for discordance at single marker loci. If only one discordant marker (a null allele or somatic mutation) is present between the maternal and fetal specimen, and concordance between the maternal specimen and the fetus at all other loci is observed, the identity of the maternal specimen can often still be reliably assured. It is recommended that assays for MCC include a sufficient number of markers to minimize the risk of mismatching a maternal/fetal pair. Inclusion of additional markers on an as-needed basis, to enhance the informativeness of the assay, may be useful in such situations.

2. Organizations such as the American Association of Blood Banks provide annual survey updates for the frequencies of null and *de novo* mutation rates among short tandem repeat and variable number of tandem repeat markers. This information could be useful in marker selection and/or reconciling an observed discordant result between the maternal and fetal alleles for any particular marker/locus. (*http://www.aabb.org/Content/Accreditation/Parentage_Testing_Accreditation_Program/Relationship_Testing_Annual_Reports/reports.htm*, last accessed August 15, 2010).

3. A high risk for misinterpretation of the result of a fetus and a biological parent occurs within the setting of a gamete donor or surrogate pregnancy. In this scenario, discordance between the maternal (ie, surrogate mother) and fetal specimen at multiple loci would be observed. Such findings should alert the laboratory to initiate further investigation(s) to determine the origin of discordance. If the information regarding a gamete donor or a surrogate pregnancy is confirmed, the MCC assay would still be valid, and the presence of any contaminating secondary maternal alleles (of surrogate origin) would still be expected to raise suspicion of sample contamination.

Reporting Guidelines

1. The prenatal diagnostic results should not be released before the MCC analysis is completed.

2. MCC assays may incidentally identify or be suggestive of the presence of a chromosomal aneuploidy. The MCC assay serves to answer a different clinical inquiry and should not be used to report the presence of an aneuploidy, or copy number variants, without confirma-

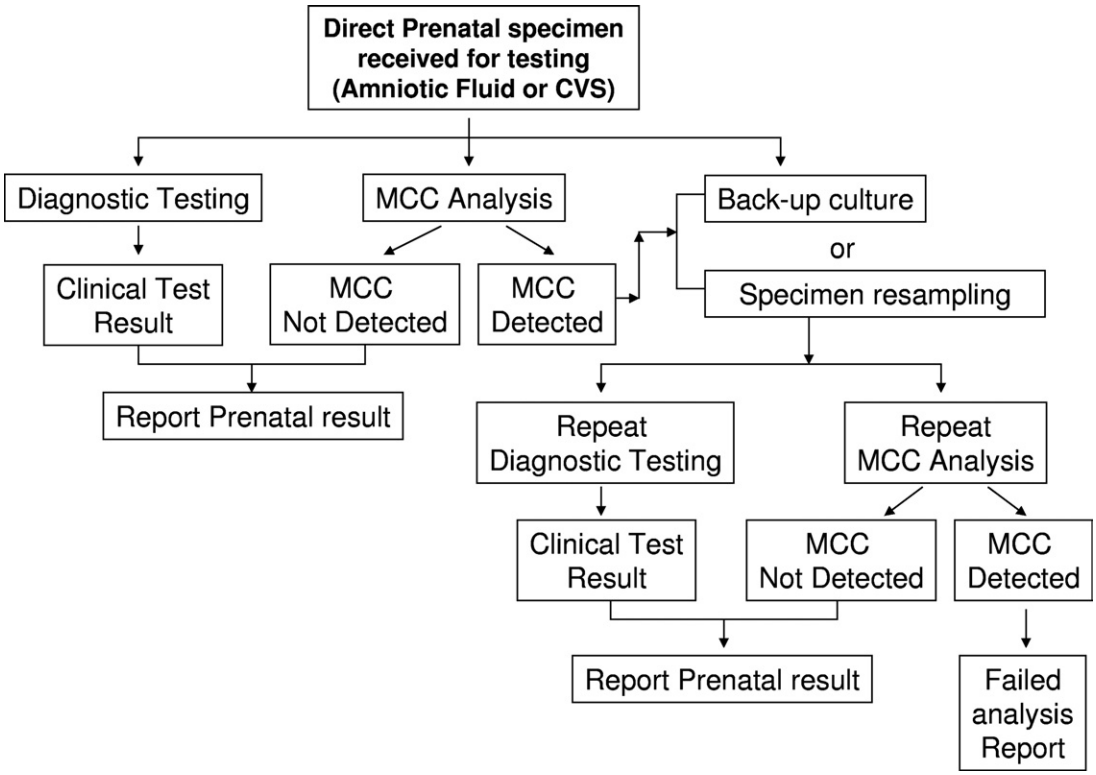

Figure 1. A recommended testing algorithm for prenatal analyses.

tion by a second method validated specifically for this purpose.

3. Multiple gestation pregnancies should be acknowledged on the report along with a caveat regarding the potential impact of fetal sampling on the accuracy of MCC results.

4. It should be acknowledged that the absence or presence of MCC in the tested specimen does not rule in/out the presence or absence of contamination in another specimen or subspecimen obtained from the fetus under consideration.

5. The sensitivity of the MCC assay should be indicated on all issued MCC reports.

6. If no MCC is detected, the accuracy of the prenatal test result can be reliably assured at the level of detection of the MCC assay. The presence of very small peaks, possibly indicating low-level MCC at or below the validated level of detection for the MCC assay or below the threshold of sensitivity of the clinical assay, may require acknowledgment on the reports. Comments in the context of such reports may include phrases such as "significant maternal cell contamination not detected," or "maternal cell contamination is unlikely to have interfered with the reported fetal result." If significant MCC is detected at a level expected to interfere with the prenatal test result, a repeat specimen should be requested, and it should be clearly stated that no result is available. Options for further analysis may be provided as appropriate. Follow-up by direct communication with the referring clinician or genetic counselor may also be considered.

7. Final prenatal test results should be reported to the referring physician in conjunction with the MCC report.

Conclusions

The guidelines provided are intended to assist laboratories in developing and optimizing prenatal MCC analysis, to provide prenatal genetic testing results of high quality and to enhance accurate result interpretation. These guidelines are expected to evolve and undergo further additions/modifications as emerging prenatal detection technologies such as circulating fetal DNA in maternal plasma and other technologies for molecular, cytogenetic, and biochemical diagnosis are developed. We recommend that MCC testing be performed on all diagnostic prenatal samples without exception and regardless of the mode of inheritance of the potentially underlying condition. Further, our recommendation is that MCC testing should be the standard of care for prenatal diagnosis using methods of molecular mutation analysis and for molecular cytogenetic applications such as array comparative genomic hybridization. Consideration of MCC studies for those CVS cytogenetic studies that do not include results from a direct culture but show a female (XX) karyotype, and for all prenatal biochemical analyte assays is strongly recommended, given the serious implications of inaccurate results in the prenatal setting. Finally, the included testing algorithm (Figure 1) could be followed or adapted to the specific capabilities and practice of clinical diagnostic laboratories.

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References

1. Macdonald F: Practice of prenatal diagnosis in the UK. Clinical Risk 2008, 14:218–221
2. Stojilkovic-Mikic T, Mann K, Docherty Z, Mackie Ogilvie C: Maternal cell contamination of prenatal samples assessed by QF-PCR genotyping. Prenat Diagn 2005, 25:79–83
3. Winsor EJ, Silver MP, Theve R, Wright M, Ward BE: Maternal cell contamination in uncultured amniotic fluid. Prenat Diagn 1996, 16: 49–54
4. Saura R, Roux D, Taine L, Maugey B, Laulon D, Laplace JP, Horovitz J: Early amniocentesis versus chorionic villus sampling for fetal karyotyping. Lancet 1994, 344:825–826
5. Schrijver I, Cherny SC, Zehnder JL: Testing for maternal cell contamination in prenatal samples: a comprehensive survey of current diagnostic practices in 35 molecular diagnostic laboratories. J Mol Diagn 2007, 9:394–400
6. Frederickson RM, Wang HS, Surh LC: Some caveats in PCR-based prenatal diagnosis on direct amniotic fluid versus cultured amniocytes. Prenat Diagn 1999, 19:113–117
7. Smith GW, Graham CA, Nevin J, Nevin NC: Detection of maternal cell contamination in amniotic fluid cell cultures using fluorescent labelled microsatellites. J Med Genet 1995, 32:61–64
8. Deucher A, Chiang T, Schrijver I: Rare sequence variation in the genome flanking an STR locus can lead to a question of "Nonmaternity." J Mol Diagn 2010, 12:384–389