Introduction

A spectrum of prenatal investigations is available to enable diagnosis of inherited disorders. To obtain material for such analyses the invasive procedures amniocentesis, chorionic villi sampling and fetal blood sampling are performed. These methods of tissue sampling can be carried out under sonographic control by specialized gynecologists who send the specimen to genetic laboratories for examination. All prenatal invasive techniques are associated with a significant procedure-related risk for abortion. This risk has to be taken into account for the individual decision of whether a prenatal genetic analysis should be performed or not.

Amniocentesis (AC)

Although AC is performed mostly between the 15th - 17th week of pregnancy, early AC (13./14. week) can be applied if a sufficient amount of amniotic fluid is available. On average, tissue culture needs 10 - 14 days to obtain enough cells for chromosome analysis. However, in case of at least 15 ml of amniotic fluid the average turn-around-time in our lab will usually not exceed 8 - 9 days after arrival. For simultaneous rapid aneuploidy screening using interphase FISH see our information “Aneuploidy Interphase”.

Methods: As a routine, four independent *in situ* cultures are initiated. According to international standards and guidelines, the cytogenetic analysis is usually based on at least 15 cells from 10 colonies out of two independent cultures.

Complications: The rate of unsuccessful culture attempts is less than 0.5%. The reliability of a normal diagnosis is sometimes restricted by undetected mosaicism and maternal cell contamination.

The risk of maternal contamination is increased in cases of complicated amniocentesis, transplacental puncture and blood contaminated amniotic fluid. To reduce the probability of maternal contamination, it is suggested to discard the first 1-2 ml of the amniotic fluid. In case of suspected contamination with maternal material, a microsatellite analysis (molecular genetic analysis) should be considered. Using this method, highly polymorphic DNA markers are used to differentiate fetal from maternal cells.

Chorionic villi sampling (CVS)

Chorionic villi sampling can be done between 10th and 12th week of pregnancy. 15-40 mg of chorionic villi is sufficient for the initiation of a two culture system for cytogenetic analysis of chorionic villi: short term culture (STC) and long term culture (LTC).

Results of STC are reported after 48 hours, while results of the LTC are completed between 6 to 8 days after arrival of the sample.

Methods: Immediately after arrival, the chorionic villi are separated from maternal tissue under a dissection microscope. If sufficient material is available, STC and LTC are initiated simultaneously. If the amount of the chorionic villus sample is too small, direct preparation, respectively STC will not be carried out and the physician informed. Setting up STC and LTC is comparable to the processing of cultures of amniocytes.

Complications: The success rate of chorionic villi is comparable to that of amniotic fluid cell culture. Mosaicism is observed in about 1% of the cases and further clarification is recommended before irreversible decisions concerning the pregnancy are made.

In the majority of cases mosaicism is restricted to the extra-embryonic tissue and does not represent the fetal karyotype, a constellation termed as “confined placental mosaicism” (CPM).

Fetal Blood Sampling (FBS)

Presently FBS by cordocentesis is possible after the 18th week of pregnancy.
Chromosome analyses can be successfully performed in 1ml of fetal blood.
The chromosome analyses with FBS has a turn-around time of 2-3 days after arrival of the sample in our laboratory.

Methods: The cultivation of fetal blood cells is comparable to PHA-stimulated cultivation of lymphocytes from peripheral blood.

Complications: Each sample has a risk of being contaminated with maternal blood cells. To prove the exclusive fetal origin of the sample an additional investigation, the so-called Kleihauer-Betke test or molecular genetic testing of polymorphic genetic loci can be arranged.

Limitation of prenatal chromosome analyses

Mosaicism: For every chromosome analysis only a limited number of cells can be examined. Therefore the existence of cellines with different karyotypes (mosaicism) concerning the fetus might go undetected.
The amount of cells analysed according to national and international guidelines detects mosaicism in the fetus with a given probability. If a higher resolution for the exclusion of mosaicism is necessary due to special clinical problems, a more extensive analysis can be requested.

Structural chromosome anomalies: The chromosomes are examined with a light microscope. The optical resolution of the light microscope is limited for physical reasons. Therefore, structural chromosomal abnormalities below a given level can not be displayed with this instrument. Furthermore, the quality of resolution for chromosome analyses depends on the contraction and spreading of the chromosomes. With the final human genetic expertise an average banding resolution is given. This represents a rough measure of the degree of contraction of the analysed chromosomes. Chromosome anomalies which comprise regions with 3-5 million base pairs and less cannot be detected by conventional chromosome analysis (microdeletion syndromes). If a microdeletion syndrome is suspected, a *fluorescence in-situ hybridization* (FISH) analysis is recommended.

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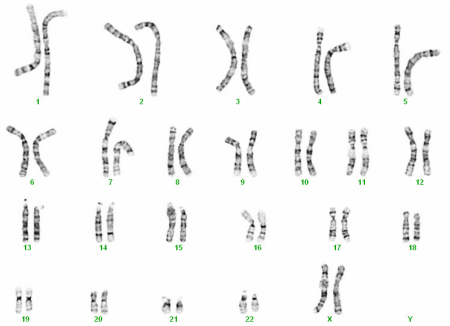
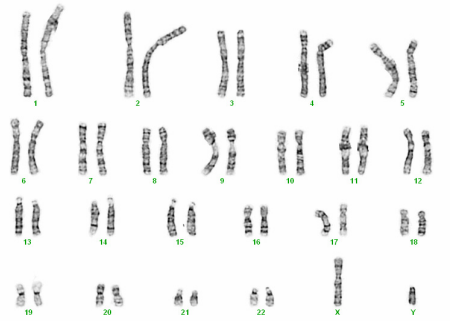
Prenatal Diagnosis – Chromosome Analyses

Technical Information



Karyogram of a numerical and structural normal female chromosome complement (46,XX)



Karyogram of a numerical and structural normal male chromosome complement (46,XY)

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