1) Limitations of cytogenetic testing (karyotyping)

Small subtle chromosome abnormalities

- Karyotyping relies on G-band quality and resolution. In general, blood samples give the best quality chromosomes and therefore provide the best chance of detecting small subtle chromosome abnormalities. Chromosomes from other tissues (e.g., amniotic fluid, chorionic villus and products of conception) give poorer quality chromosomes, hence the risk of missing a subtle abnormality is increased.

- It should be noted that on rare occasions a subtle abnormality may be missed at prenatal diagnosis, only to be diagnosed later on a postnatal blood sample. A policy statement to this effect has been issued by the Association of Clinical Cytogenetists (ACC).

- It should also be understood that even a G-band blood karyotype can never exclude extremely subtle chromosome abnormalities that are at the limit of resolution of light microscopy. Microarray testing should be performed in these cases where the patient meets the appropriate criteria.

- The Laboratory adheres to national professional standards for the minimum acceptable banding resolution for specified types of clinical referral. If a repeat sample is required due to analysis failing to meet the minimum standard, this will be stated on the report.

Detection of mosaicism

- Although mosaicism may be detected by routine karyotyping it can never be 100% excluded. However, if there is an indication of suspected mosaicism, additional cells will be examined to exclude 10% mosaicism at a 95% confidence level.

Interpretation of mosaicism in prenatal diagnosis

- True mosaicism, when detected prenatally, can be difficult to interpret and a further invasive diagnostic test may be required. Mosaic cell lines may be unevenly distributed between the fetus and extra-fetal tissues leading to false positive and false negative results in the most extreme cases. ‘Confined placental mosaicism’ (CPM) is observed in approximately 1-2% of CVS samples.

- Pseudomosaicism can arise as an artefact of culture and is not representative of the fetal karyotype. This is normally present in only one of two or three independently established cultures and can therefore be interpreted accordingly. In most cases, no further invasive testing is required.

Maternal cell contamination in prenatal diagnosis

- Maternal cell contamination of chorionic villus and amniotic fluid occurs in approximately 1/250 samples and may occasionally complicate the interpretation of results.

Normal variation

- Each chromosome pair has a specific and identical G-banding pattern in all individuals. However, variation of no clinical significance may occur around the centromeric regions and short arms of some chromosomes. These variations are known as ‘polymorphic variants’, ‘polymorphisms’ or ‘normal variants’.
• In a few cases, it is necessary to distinguish between such variation and a true abnormality. It may therefore be necessary to karyotype the parents or to carry out further tests on a repeat sample.

2) Limitations of cytogenetic testing (FISH)

FISH (Fluorescence in-situ hybridisation)

• FISH can only detect deletions or duplications of regions specifically targeted by the probe used and which are larger than the probe used. It is possible that rare very small deletions may not be detected by FISH.

3) Limitations of cytogenetic testing (Microarray testing)

CGH Microarray testing (array CGH)

• Array CGH can detect copy number change (deletion or duplication) in the genome at a higher resolution than G-band analysis. The technique involves a comparison of patient DNA with reference DNA from normal individuals and because of natural copy number variation, detected copy number change may represent normal variation either in the patient or the reference DNA.

• Currently our diagnostic array platform (Roche-NimbleGen 12x135K allows us to detect copy number changes of 200kb at a 95% confidence level and 100kb copy number changes at a 80% confidence level. This is well within the proposed recommended minimum resolution required by Best Practice Guidelines.

• Array CGH cannot identify balanced structural changes in the chromosomes, and may not detect mosaicism.

• For these reasons positive CGH microarray findings are usually followed up by FISH analysis. FISH can confirm if an array result is clinically significant and can also detect carriers of balanced chromosome abnormalities. FISH testing may need to be extended to the patient's parents to refine the clinical interpretation of the microarray results.

4) Limitations of cytogenetic testing (MLPA)

MLPA (Multiple Ligation-dependent Probe Amplification)

• Like array CGH, MLPA detects copy number change and interpretation of results can be complicated by natural copy number variation. The probes or probe kits used however have been selected and validated to reduce the likelihood of false positive or negative results.

• Like array CGH, MLPA cannot detect balanced chromosome rearrangements.

• MLPA can only detect changes at the site of the probe used therefore MLPA will not be able to detect telomeric deletions/duplications in region not identified by the MLPA probes used.