

Implementation of QF-PCR and MLPA analysis for fetal and neonatal pathology samples.

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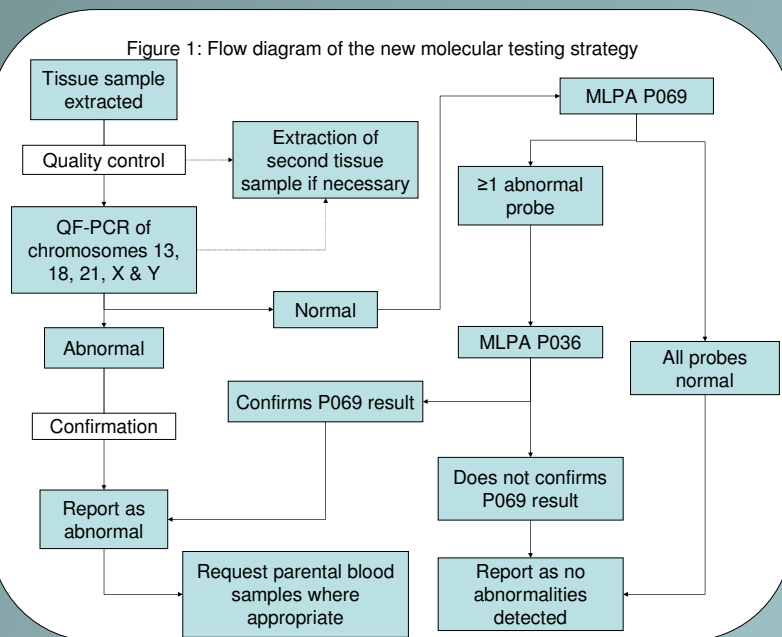
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Introduction

Chromosomal abnormalities are a significant cause of pregnancy loss, causing more than 50% of early miscarriages ⁽¹⁾. Stillbirth, intra-uterine death, neonatal death, and structural or congenital malformations may also be associated with cytogenetic abnormalities ⁽²⁾. Identification of the cause of pregnancy loss is important for establishing recurrence risk and management of future pregnancies.

Solid tissue fetal and neonatal pathology samples have traditionally been analysed by cell culture and karyotype analysis, however there is a high failure rate due to the often poor condition of the tissue received, and this approach is labour intensive and expensive. In our laboratory we observed a 22% failure rate, with other groups having rates of up to 30% ⁽⁴⁾.

Following a retrospective audit and a validation period we decided to implement a QF-PCR and subtelomere MLPA approach for diagnostic testing of fetal and neonatal pathology tissue samples. This approach has been reported to be less labour-intensive, allows a higher sample throughput, and has a higher success rate than karyotype analysis ^(3,4,5).



Molecular Testing Strategy

The new QF-PCR and subtelomere MLPA testing strategy is outlined in figure 1. Cytogenetic analysis is still carried out when there is a known familial chromosome rearrangement or mosaicism is specifically queried. FISH testing for DiGeorge/Velocardiofacial syndrome region (22q11.2) deletions is performed in referrals where there is a cardiac anomaly. Cell culture may also be necessary for some cases, for example where the diagnosis of a metabolic disorder is suspected.

1st year of Diagnostic testing

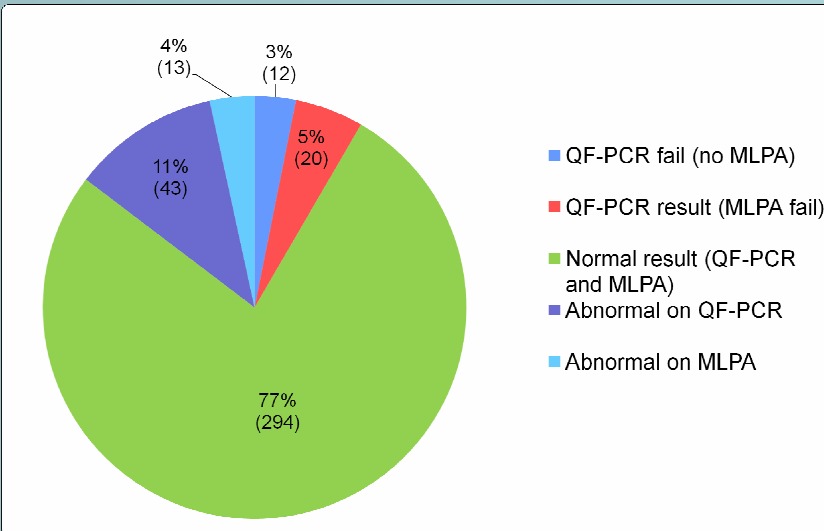
During the first year of molecular testing 382 samples were analysed. The results are summarised in figure 2. Only 12 (3%) of samples failed to give a result on QF-PCR and therefore did not undergo MLPA analysis. This is in contrast to the 20-30% failure rate observed with cell culture and cytogenetic analysis. A full result was obtained for 350 samples, of these 56 (16%) gave an abnormal result (see table 1). As expected the majority of abnormal results (77%) were detected by QF-PCR.

Seven poor quality tissues were analysed which would not have been suitable to attempt culture and cytogenetic analysis. Two of the samples showed an abnormal result on QF-PCR analysis and a further sample gave an abnormal result on MLPA.

Table 1: Summary of abnormal results

Abnormal result	Number	%
Triploid	11	19.6%
Trisomy 18	11	19.6%
45,X	10	17.9%
Trisomy 21	9	16.1%
Trisomy 13	2	3.6%
Trisomy 16	2	3.6%
Trisomy 17	1	1.8%
Trisomy 7	1	1.8%
Trisomy 9	1	1.8%
Reciprocal subtelomere imbalance	1	1.8%
Single subtelomere imbalance	6	10.7%
Double subtelomere imbalance	1	1.8%
Total	56	

Figure 2: Summary of QF-PCR and subtelomere MLPA results for first year of diagnostic testing.



Conclusions

QF-PCR and subtelomere MLPA is an effective testing strategy for analysis of fetal and neonatal pathology samples, with many advantages over conventional cytogenetic analysis. Cell culture and chromosome analysis from fetal and neonatal pathology samples is difficult, labour intensive and time-consuming. In our hands the new strategy has improved our success rate considerably. In particular, some tissues would not be accepted for cytogenetic analysis e.g. macerated, decomposing, very old (more than 10 days), or very small in size. DNA extraction and molecular genetic analysis, particularly QF-PCR, is often possible for these specimens, and therefore a result may be obtained where previously this was not possible. A further advantage of this method is that DNA can then be stored for future analysis if appropriate, such as array-comparative genomic hybridisation (CGH). In conclusion, we would recommend this approach to other laboratories.

References

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