Introduction

The Cell3™Direct technology is the first commercially available solution that enables non-invasive prenatal determination of fetal RhD genotype and RhesusD (RhD) genotyping by quantitative PCR (qPCR). This technology (Nonacus, UK), which enables qPCR testing to be conducted directly from plasma, thus avoiding the time consuming and costly necessity of cfDNA extraction.

Methods

**Samples:** Samples containing RhD positive genomic DNA (gDNA) spiked in RhD negative gDNA; and male gDNA spiked in female gDNA at different ratios were used for initial assay evaluation. 100 plasma samples from RhD negative pregnant women (24-26 weeks gestation) and 50 samples from healthy pregnancies (10-27 weeks of gestation) were used to assess the sensitivity and specificity of this technology.

**qPCR:** The Cell3™Direct: Rhesus D Fetal Blood Group Genotyping kit was used to investigate the fetal RhD genotype in the 100 plasma samples from RhD negative pregnant women. The kit targets sequences specific for exons 5, 7 and 10 of the RhD gene and therefore can distinguish between fetal RhD positive, negative and RhD PSI genotypes in a background of RhD negative maternal DNA. A target for the CCR5 control gene is also included to confirm adequate cDNA quantities within the sample. The Cell3™Direct: Fetal Sex Determination kit was used to determine the fetal sex in the 50 plasma samples from healthy pregnancies. The kit detects the Y-chromosome specific genes SRY, TSPY and DAZ; as well as the CCR5 control gene.

**Data Analysis:** The samples were blinded and analyzed independently using parameters comparable to those used routinely in a clinical setting.

Workflows advantages of testing direct from plasma

The routine workflow used in current practice for NIPD of fetal RhD genotype and fetal sex determination requires cfDNA extraction, resulting in a sample to result turn-around-time of 4-5 hours.

- **Blood sample**
- **Plasma isolation**
- **cfDNA extraction**
- **qPCR testing**

By using the direct from plasma approach, cfDNA extraction is no longer required, which results in a faster and less expensive workflow with a sample to result turn-around-time of 2.5-3 hours.

With the streamlined workflow of the Cell3™Direct: Rhesus D Fetal Blood Group Genotyping kit, sample setup requires only three simple steps.

References


Validation on clinical samples

Testing on the 100 plasma samples obtained from RhD negative pregnant women at 24-26 weeks of gestation was conducted using the Cell3™Direct: Rhesus D Fetal Blood Group Genotyping kit. Fetal RhD genotype was known for the entire cohort, with 68 RhD positive and 32 RhD negative outcomes. Results showed sensitivity and specificity rates of 100%, with a 2% inconclusive rate, a 4% repeat rate and a 0% failure rate. Testing on 50 plasma samples from healthy pregnancies at 10-27 weeks of gestation (average gestation of 15.3 weeks) was conducted using the Cell3™Direct: Fetal Sex Determination kit. Fetal sex was known for all samples, with 25 male and 25 female outcomes. Results revealed sensitivity and specificity rates of 100%, with a 2% repeat rate and a 0% inconclusive and failure rates. Test setup and analysis was conducted blinded for all samples.

Conclusions

The Cell3™Direct technology is the first commercially available solution that enables Non-Invasive Prenatal Testing of fetal RhD genotype and fetal sex determination direct from plasma. These kits set a new benchmark in simplicity of setup with results generated within 3 hours. The protocol offers significantly reduced hands-on time due to no sample extraction requirement and flexible pre-plated assays. Additionally, the total plasma quantity required is 80 μl per sample, therefore allowing for a smaller volume of blood to be drawn and plenty of sample remaining for further tests to be undertaken.

Technical evaluation

The accuracy and sensitivity of the Cell3™Direct: Rhesus D Fetal Blood Group Genotyping kit was evaluated on artificially prepared samples containing RhD positive gDNA spiked into RhD negative gDNA at 10% and 1% ratios. A 10% Rh D PSI variant spike-in sample was also tested to prove that the exon 5 target could discriminate it from the RhD wild type sequence. Similarly, the Cell3™Direct: Fetal Sex Determination kit was evaluated on samples containing male gDNA spiked into female gDNA at the same ratios. Mean Cq values were calculated from 3 replicates per target and 1 ng of total DNA was used per replicate.