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The need of short turnaround times for whole exome sequencing in fetuses with multiple congenital anomalies on ultrasound

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Library preparation for next generation sequencing remains a difficult bottleneck in clinical settings. Although there are automation systems available for higher throughput labs, clinical settings often have fewer samples, but critically require faster turnaround time of results, making these systems costly and inflexible. Here we tested the automated library preparation of Agilent's Magnis NGS Prep system on WES trio's and measured the reduction of hands-on time and turnaround time.

BACKGROUND:

In prenatal diagnosis, identifying the cause of anomalies seen on fetal ultrasound provides important information for pregnancy and improves perinatal management options (fig 1). The conventional test (QF- PCR and microarray) leads to a diagnosis in approximately 25% of cases with multiple congenital anomalies (MCA) on fetal ultrasound. The introduction of whole exome sequencing in 2017 (fig 2) improved diagnostic yield to 63%, with a turnaround time of 8-9 days (range of 6-14). (Corsten- Janssenet al, 2020)). However, implementing WES in prenatal setting is challenging due to uncertainties around fetal phenotyping, variant interpretation and incidental findings; required short turnaround times and ethical issues. Though, anything that helps to reduce the time to answer must be considered. Therefore, we tested the Agilent's Magnis NGS Prep system automation system to investigate if the turnaround time of the WES procedure could be further reduced.

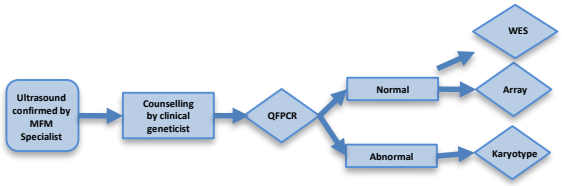

Figure 1: The workflow of how anomalies were further investigated once detected by ultrasound. Our current method now has the addition of a WES alongside array testing once a normal result is obtained via QFPCR.

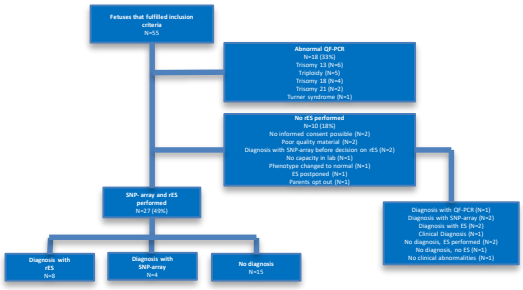

Figure 2: The results of a study with 55 cases were 18 abnormalities were detected with QFPCR. Further investigation of 27 with WES resulted in 8 with clinically relevant results. A further 4 were detected with the array but not by sequencing. Hence, the need for NGS and arrays needing to be used alongside each other.

METHODS:

A sample set of (N=32) were anonymized and were run on the Magnis. A subset of 2 samples were also run on the Bravo system and the results were subjected to the same analysis inhouse bioinformatics pipeline to detect any differences as a result of the sample preparation instrument.

Whole Exome Sequencing using Bravo automation.

- Agilent SureSelect QXT Sample preparation.
- Input 50ng of high-molecular weight DNA.
- Capturing with the Agilent SureSelect Exome_v6 baits
- The samples were pooled equimolar and 1.3 pM was loaded on the Nextseq High output kit for sequencing.

Whole Exome sequencing using Magnis. (A total of 4 runs)

- Agilent SureSelect XT HS library preparation.
- DNA input 50ng of high-molecular weight DNA, which were fragmented with Agilent fragmentase enzyme in a thermocycler.
- Capturing with the Agilent SureSelect Exome_v7 baits (v6 is not compatible).
- The samples were pooled equimolar and 1.5 pM loaded on the Nextseq High output kit for sequencing.

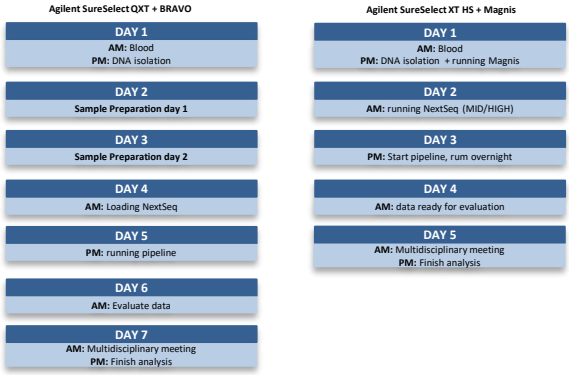

Figure 3: Schemtic workflow of the Bravo vs Magnis system
The comparison of the major steps required using the SureSelect QXT + Bravo versus the SureSelect XT HS +Magnis. The initial tests showed promise to implement a workflow that can reduce turn around time of our samples by two days.

RESULTS:

The initial runs with the Magnis System produced NGS libraries in 5 days, compared to the 7 days that it takes with the Agilent Bravo method (figure 3). NGS sequencing data were of comparable quality with both methods. The relevant variants called from both methods were the same. Any differences of other variants were investigated and were shown to be an artifact.

DISCUSSION:

Although the department is already equipped with automation to speed up the library preparation, our first runs show that the Magnis is able to reduce this preparation by a further two days. This potential reduction of turn around time makes the Magnis system very attractive to use in prenatal WES workflow. Further testing that still needs to be done to ensure the data produced is robust and this workflow can be implemented.
Our first experience has been positive with the instrument being easy to use, with a reduction in manual steps meaning a reduction in possible manual errors.
There are some limitations to work around since only a 8 sample format is available, and no possibility to combine different library preparation procedures.
However, the possibility of this automation system that would allow for quicker turnaround of critical samples will be a big step forward for any department with the same need.

Corsten- Janssen et al 2020; Prenatal Diagnosis accepted for publication