relevant. Therapy was initiated before release of results in 27%, after cytogenetics in 9%, after FISH in 3%, after array in 1% and supportive care in 60%. The preliminary data suggests a tier testing approach does not compromise patient care if tier testing is completed within 3-4 weeks of initial diagnosis. In presence of unfavorable prognostic abnormalities by cytogenetics, array testing may not be warranted. Based on this data, we would like to propose the optimal genetic testing algorithm.

P14.26-M
Real-time and Droplet PCR quantification for non-invasive determination of RHD incompatibility between mother and foetus

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The aim of this study was to compare two strategies of DNA quantification: Real-Time PCR (qPCR) and Droplet PCR (dPCR). The benefit of dPCR against qPCR is represented by absolute quantification of target nucleic acid molecules without the requirement of calibration curves. The methods were compared by quantification of standard nuclear DNA of known concentration. DNA was eightfold diluted (2-0.015 ng/ul) and twelve replicates were realized for each dilution. Concentrations were then measured as levels of amplicons of two genes, housekeeping gene GAPDH and human RHD gene (exon 10). The same genes were analyzed in plasma cell-free DNA and also in cell-free fetal DNA isolated from maternal plasma. Evaluation of these two methods' performance was based on several parameters, such as degree of linearity (R^2), accuracy, detection limit, etc. In case of standard nuclear DNA, both methods showed high accuracy and low detection limit, both genes were detected even in most diluted samples. High linearity degree (R^2 > 0.99) has been reached by both methods. The correct RHD status of tested women was immunologically verified. Regarding analysis of cell-free fetal DNA in RHD negative maternal plasma, dPCR failed against the qPCR to convincingly distinguish RHD positive samples from the negative ones. For the purpose of prenatal screening, the qPCR method would be probably the preferable alternative due to very low fetal DNA concentrations (about 0.002 ng/ul) in maternal plasma, which is under the detection limit of dPCR.
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P14.27-S
Development and evaluation of a gene panel for the diagnosis of monogenic epilepsies

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Next Generation Sequencing (NGS) is a great advance in the field of molecular genetics, especially for diseases with high genetic heterogeneity, allowing parallel testing of many disease-causing genes. It will lead to a better access of a larger number of patients to molecular diagnosis. In order to improve the diagnostic yield of genetic testing in patients with epileptic diseases, we developed a panel including 41 genes causing monogenic form of epilepsy or neurodevelopmental disorders frequently associated with seizures. Until now, only 13 of these genes have been currently screened by Sanger sequencing, on a sequentially basis. In the present study we compared two methods: the Haloplex technology (Agilent) and the SeqCap (Roche) technology. Sequencing was performed using an Ion Torrent PGM sequencer (Life Technologies). We performed NGS sequencing on DNA samples from 44 patients, including 38 patients in whom a (probably) causative mutation had been previously found by sequential Sanger sequencing in our routine practice, and 6 novel patients who had not been screened. DNA samples were anonymized and the first steps of the analysis were performed following a blind protocol. DNA from all the patients was analyzed with the Haloplex technology and only 24 of them were also analyzed with the SeqCap technology (they were sorted among the 44 patients). Data analysis will be performed with the NextGene Software (Softgenetics) and also with specific BWA-GATK pipeline based on the recommendations of BroadInsitute. Our analysis will include coverage, sensitivity and false positive rate as well as a cost evaluation.

P14.28-M
Scaling up whole-exome sequencing on Ion Proton using AmpliSeq

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The Ion Proton[a] and Ion PGM[a] (Life Technologies) technologies are used in the National Genomics Infrastructure - Sweden (NGI) to handle the different requests and needs for rapid massively parallel sequencing, (MPS).
Ion Proton[a] enables a rapid workflow for human whole exome sequencing. The PCR based whole exome capture (Ion Ampliseq Exome Kit) provides consistent high coverage across annotated exonic regions, with the PI[a] chip yielding 50-60.000 SNPs per sample, with ~98% of these overlapping variants reported in dbSNP. Sample preparation and sequencing is performed in less than two days. We are exploring the possibility to scale-up the throughput by loading two PI chips on one initialization and to combine 3 exomes per PI chip. With this set up it will be possible sequence up to 30 exomes per week on one Ion Proton[a].
The bioinformatics analysis has been streamlined using an in-house database system, based on R and MySQL, where all detected variants from all in house exome-sequencing runs are stored. This system allows for very efficient and fast filtering of SNPs or indels between any groups of samples and we currently have a success rate of almost 80% of finding disease-causing variants in small families or trios with rare Mendelian disorders.
Ion Proton[a] is also being used for clinical applications such as identification of fusion transcripts from cancer samples, mutation screening using panels of candidate genes. The sample preparation will be simplified and faster when the automated system, Ion Chef[a] is introduced and established in the workflow.

P14.29-S
Evaluation of three sequence capture platforms for whole exome sequencing

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Whole exome sequencing (WES) can be effective for identifying sequence variants. Here, we present a comprehensive comparison of the most recent next generation sequencing (NGS) exome enrichment methods of Agilent (SureSelect V5+UTR), NimbleGen (SeqCap V3+UTR), and Illumina (Nextera Expanded Exome). Exomes of six human DNAs were captured by these methods and sequenced at 100x depth of coverage on an Illumina HiSeq 2000 platform by four vendors. Read depth, % of coverage, GC bias, and number of detected single nucleotide variations (SNVs) and small indels were compared. To examine the methods' ability to identify SNVs and small indels, we analyzed heterozygous positions and small indels previously detected by Sanger sequencing (SS). For two DNAs, WES data were also compared to whole genome sequencing (WGS). SureSelect and NimbleGen demonstrated highest average read depth in target region. Considering ≥20x for read depth, SureSelect covered the largest proportion of its targeted bases. SureSelect and NimbleGen showed the highest average read depth as well as detected the highest number of SNVs and small indels in RefSeq. However, all SNVs identified by SS were accurately called by all three methods. Less consistent was the detection of small indels, where a substantial difference among the platforms and vendors was observed. In addition, all three methods showed high GC bias which was not seen in WGS. Our analysis indicated a considerable variability among exome enrichment methods, DNA sequencing laboratories, and even among DNA samples. Our data revealed that both SureSelect and NimbleGen performed better than Nextera.

P14.30-S
The importance of root cause analysis following an external quality assessment (EQA) to improve the quality and accuracy of a diagnostic service

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EQA is essential to verify the quality and accuracy of the diagnostic service. When new technologies emerge, EQAs need to be developed to verify the laboratory diagnostic validation process and enable benchmarking of their performance. When laboratories introduce new technologies diagnostically, most critical errors in EQAs are analytical. As laboratories gain more experience in the new technologies, the critical errors usually become limited to the interpretation of results.