HTG Molecular

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AG13.13 - Nuclease protections assay zum Nachweis von Genfusionen

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Background
Gene fusions play a particularly important role as diagnostic or predictive biomarkers. Common methods for detecting rearrangements are FISH, immunohistochemistry or DNA- or RNA-based sequencing methods. Nuclease protection assays provide the ability to detect gene fusion multiplexing. As part of a pilot project, we tested the method with regard to the detection of ALK, ROS1 and RET translocations. The results were compared with previously obtained data from FISH studies, immunohistochemistry and sequencing methods. Furthermore, the feasibility of the method was examined by means of preanalytical variables (tissue size, relative tumor cell content, age of paraffin blocks and sections, decalcification, etc.).

Methods
A total of 96 tissue samples were analyzed, including 31 ALK, 9 ROS1 and 6 RET positive lung cancers. The tissue sizes varied from 0.5 to 200 mm 2. The tumor cell content was between 10 and 80%. Up to 9 years old blocks and up to 5 years old archived blanks were used. The materials were analyzed using the HTG Edge Seq ALKplus System. Tissue samples are merely lysed (without separate RNA extraction) and processed in the EdgeSeq processor. Tagging and adapter ligation complete the Library Prep. Subsequent analysis was performed on the Illumina MiSeq. The bioinformatic evaluation was carried out with commercial parsing software and own analyzes.

Results
The tested assay also gave valid results on diagnostically difficult materials, e.g. at very low material usage - up to 1.5mm2. The age of the paraffin blocks and the slice preparations had no negative impact on the feasibility, as long as the tissue samples were fixed in buffered formalin. Even decalcified tissue could be successfully analyzed. Overall, the concordance rates for the previous analyzes were 100%, the Positive and Negative Predictive values were 100% (in a preliminary analysis).

Conclusion
The HTG Nuclease Protection Assay investigated here represents an interesting alternative for the detection of gene fusions in multiplex format. The method can be well integrated into molecular pathology laboratories. Time expenditure and tissue consumption are to be regarded as favorable. It is also positive that older materials can be used. Further validation and concordance analysis with previously established biomarker assays are still required.