NTRK Gene Fusion Detection

Comparison of Available Technologies




	NGS	IHC	FISH	RT-PCR
Ability to detect different fusions	**Detects fusions in all three NTRK genes**[3,6] NGS is a sensitive and specific way to detect all the possible variations of NTRK gene fusions and also identify other bioimarkers in one comprehensive molecular test.	**May detect proteins encoded by NTRK genes but requires confirmation by other tests**[2,4,6,9] To be used as a stratifying tool; other methodologies are required to confirm gene fusions. Pan-TRK antibodies may detect presence of protein encoded by any of the three NTRK genes but can not differentiate between wild type and fusion proteins[2,4,9]	**Can detect NTRK fusions, depending on the probes used**[11,12] Different probes are required for each NTRK gene. For ROS1 fusion detection dual colour 'break-apart' probes are used which label the 3' (centromeric) part of the fusion breakpoint with one colour fluorochrome and the 5' (telomeric) part with another. Rearrangements are determined by looking at the pattern of fluorochrome expression.[6,13]	**Requires multiple primer sets to detect gene fusions**[11,14] As the location of gene rearrangements is not known at the time of testing, multiple primer sets are required, one for each fusion variant.[11,13,14]
Reliability	**Reliability varies depending on the assay used**[10] Different NGS panels target different regions of the sequence and depth of coverage varies between assays. Requirment for tumor content can also vary between assays.[10]	**Detects NTRK proteins with high specificity and sensitivity**[2,4] Pan-TRK antibodies have been reported to have 95–100% sensitivity and up to 100% specificity for TRK proteins depending on the threshold used to define positivity.[2,6,7]	**False positives or negatives might occur**[6,11] Reliability depends on the probes used. There is a risk of false-positive results due to complex chromosomal translocations and detection of non-functional fusion proteins.[6,11] Variant or complex rearrangements may be missed.[1,2,6,11] False negatives results may be above 30% in some cases.[11]	**Reliable for known gene fusions, requires FISH confirmation when testing for NTRK fusions**[7] Each variant requires a specific primer set. Therefore, PCR may miss unknown or untested variants.[5,6,13,14] ROS1 expression is detectable in normal lung tissue, PCR testing is not specific enough to detect ROS1-positive lung cancer without FISH confirmation.[8,14]
Advantage	**Can detect novel fusion partners and evaluates multiple actionable targets**[11] Depending on the assay used, NGS can evaluate multiple and novel fusion partners while preserving limited tissue. RNA sequencing is focued on coding sequences rather then introns, and is suited to gene fusion detection.[4,11]	**Low cost and readily available**[2,6,11] Pan-TRK antibodies detect TRK A, B and C proteins with a turnaround time of 1–2 days.[11]	**Readily available** Allows visualisation of the target within the cell and enables several targets to be detected in one sample using multiple fluorophores.[7,11] The use of break-apart probes allows fusions with unknown partners to be detected.[11]	**Low cost per assay with high sensitivity and specificity**[11]
Disadvantage	**May not identify all NTRK gene fusions**[11] DNA-NGS is limited by intron size. RNA-NGS is suited to gene fusion detection but may be limited by RNA quality.[4,11]	**Cannot reliably differentiate between normal protein expression and proteins resulting from gene rearrangements**[6,11] IHC can detect both wild-type and fusion TRK proteins leading to possible false positives. There is also a risk of possible false negatives for fusions involving TRKC. Therefore, orthogonal techniques are required to confirm the presence of gene rearrangements.[6,11]	**Conventional FISH may require multiple tests to detect NTRK fusions** The target sequence must be known for conventional FISH and three separate tests are required for NTRK1, NTRK2 and NTRK3.[11] Depending on the type of translocation there is also a risk of false positive or false negative results.[6,11]	**Can only detect known target sequences**[11] Target sequences must be known and cannot readily detect novel fusion partners. A comprehensive multiplex reverse transcriptase polymerase chain reaction (RT-PCR) assay might be challenging because of the potentially large number of possible 5' fusion partners.[7,11]

NGS = next-generation sequencing **IHC** = immunohistochemistry **FISH** = fluorescence in situ hybridization
RT-PCR = reverse transcription polymerase chain reaction

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