Multiplex fast FISH assay for detecting ROS1, RET and MET aberrations in FFPE specimens using BioView image analysis

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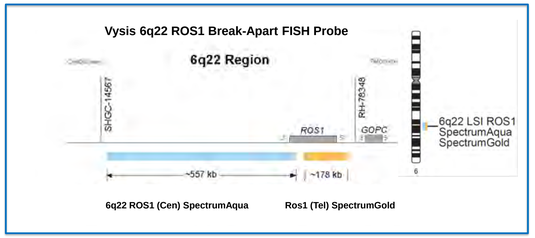
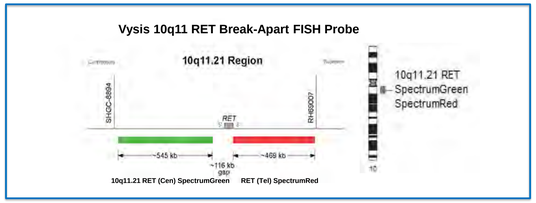
INTRODUCTION

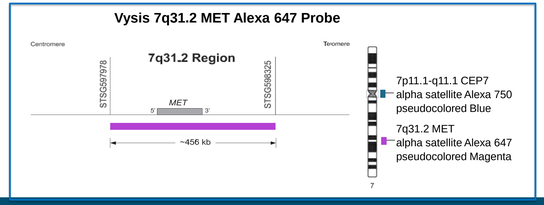
Biomarker testing in lung cancer is often limited by a lack of sufficient formalin fixed, paraffin embedded (FFPE) tissue for comprehensive genomic profiling. To promote personalized therapy for lung cancer, a multiplex FISH assay was developed to simultaneously assess aberrations in *ROS1*, *RET*, and *MET* on a single FFPE specimen slide.

DESIGN OF DNA PROBES for ROS1, RET and MET GENES

- A probe mix contained 6 differentially labeled fluorescent probes: 3' *ROS1*, 5' *ROS1*, 3' *RET*, 5' *RET*, *MET* and *CEP7*.
- The probes were formulated in Vysis IntelliFISH Hybridization Buffer to allow for a 2 h hybridization time.







MATERIALS AND METHODS

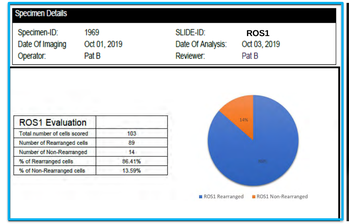
- Specimens included lung primary tumor (N=47) as well as biopsies from a variety of metastatic sites (N=16) and 1 cell line.
- The hybridization of the fluorescently labeled DNA probes to the cellular DNA site was visible by direct detection using fluorescence microscopy on BioView imaging platform.
- BioView Duet scanning system was used to perform automated slide imaging and digital analysis.

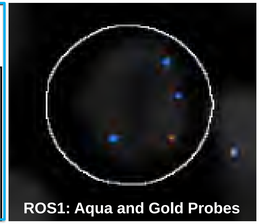
- There were 46 samples with *ROS1* rearrangements, 15 samples with *RET* rearrangements, and 25 samples with *MET* amplification reported by a previously validated laboratory test method.
- Specimens were considered positive for *ROS1* rearrangement if ≥15% evaluated cells contained a break apart (rearranged) signal.
- Specimens were considered positive for *RET* rearrangement if ≥15% evaluated cells contained a break apart (rearranged) signal.
- Specimen was considered positive for *MET* abnormality if either criteria was met:
  - ≥20% of cells were amplified, i.e. had *MET*/CEP7 ratio ≥2
  - ≥20% of cells were highly polysomic, i.e. had 5 or more *MET* signal copies per cell but *MET/CEP7* ratio was <2
  - Mean number of *MET* copies in all scored cells was ≥5
  - Mean ratio of *MET*/CEP7 in all scored cells was ≥2.0

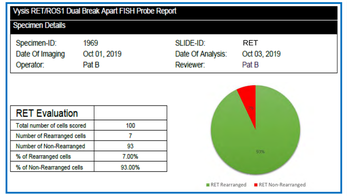
RESULTS

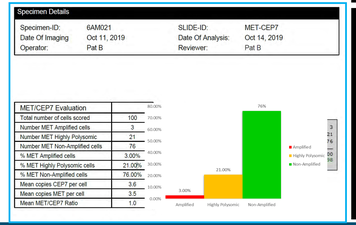
- Assay was successful in 63/64 cases for a 98.4% validity rate*.
- Hybridized slides were imaged on BioView in 6 colors corresponding to the colors of the 6 fluorescently labeled probes.
- Resulting images were automatically organized into 3 separate scan files– one for ROS1, one for RET and one for MET probes.
- User reviewed the scans and selected cells of interest which were automatically classified as positive or negative by the BioView software.
- BioView created a gallery of positive and negative cells and generated reports for each aberration.
- The information from the case reports was used for data analysis.

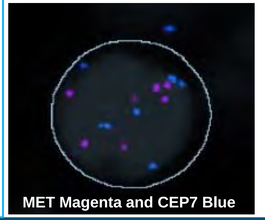
** 1 case did not have enough scorable cells.*
Note: For 7 cases hybridized slides were stored frozen for 6 weeks before image analysis. These slides had weaker but readable fluorescent signal.











The disposition of the samples tested in this study by the 6-color assay with automated imaging evaluation was compared with the disposition obtained by the previously validated test.

	Overall Percent Agreement (number of cases)	Positive Percent Agreement, (number of cases)	Negative Percent Agreement (number of cases)
ROS1	93.5% (43/46)	82.4% (14/17)	100.0% (29/29)
RET	100.0% (15/15)	100.0% (3/3)	100.0% (12/12)
MET	88.0 (22/25)	81.8% (9/11)	92.9% (13/14)

Main assay characteristics were compared between negative and positive cohorts for *ROS1, RET and MET* biomarkers.

Average Percent ROS1 rearranged cells (min-max)	
ROS1 Negative cohort, n=13	ROS1 Positive cohort, n=29
5.1% (0-13.7%)	63.1% (15.3-95.1%)

Average Percent RET rearranged cells (min-max)	
RET Negative cohort, n=3	RET Positive cohort, n=12
4.5% (0-9.9%)	50.7% (21.6-77.5%)

	MET Assay characteristics	
Category	MET Negative cohort, n=13	MET Positive Cohort,
MET Amplified cells	6.7%	15.3%
Polysomic cells	7.6%	47.9%
Average MET Copy Gain Number	3.0	4.4
MET/CEP7 Ratio	1.0	1.2

CONCLUSIONS

- A newly developed 6-color FISH assay allows simultaneous detection of three genomic abnormalities using only 1 specimen slide.
- This feature combined with rapid hybridization in IntelliFISH buffer and automated BioView slide imaging and analysis can significantly increase the yield of molecular testing on limited lung cancer tissue samples.
- Careful pathologic correlation for tumor cell identification and careful assessment of hybridization quality are necessary to optimize the accuracy of this test method.

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