

# Detection of Esophageal Adenocarcinoma (EAC) by a Four-Probe Fluorescence in situ Hybridization (FISH) Assay

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## Introduction

- Histopathologic grading of dysplasia in Barrett's esophagus shows substantial inter- and intraobserver variability
- Cell biologic markers may provide additional information regarding risk of malignant degeneration
- The purpose of this pilot study was to assess the viability and performance characteristics of a four probe FISH assay for detection of esophageal adenocarcinoma among esophageal biopsy specimens

## Methods

- Study Design:** A four-probe fluorescence in situ hybridization (FISH) assay was performed on esophageal biopsy specimens in a training set. The assay was then validated in a second set of specimens
- FISH Assay:** Assessed for DNA abnormalities in MYC[8q24], p16[9p21.3], HER2[17q11.2], and ZNF217[20q13.2]. Assays results quantified by the percentage of cells with:
  - Multiple probe gains**
  - Single probe gains**
  - Homozygous 9p21 loss**
  - Any probe gains with any 9p21 loss**
- Esophageal biopsy specimens:** Formalin-fixed, paraffin-embedded
- Training Set:** 18 normal squamous epithelium (NSE), 18 esophageal adenocarcinoma (EAC)
  - Median age 63 years, 53% male (19/36)

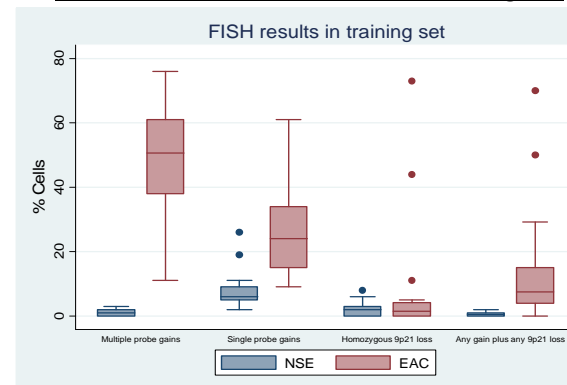
- Validation Set:** 33 non-dysplastic Barrett's esophagus (NDBE), 21 EAC
  - Median age 64 years, 93% male (50/54)
- Statistical analysis:**
  - Median assay results compared among groups using non-parametric tests (Wilcoxon Rank-Sum Test)
  - Thresholds for neoplasia detection selected in the training set by receiver operating characteristic (ROC) analysis to achieve 90% specificity and maximum sensitivity
  - Thresholds were applied to results from the validation set to calculate area under the ROC curve, sensitivity, and specificity for each test measurement

## Results

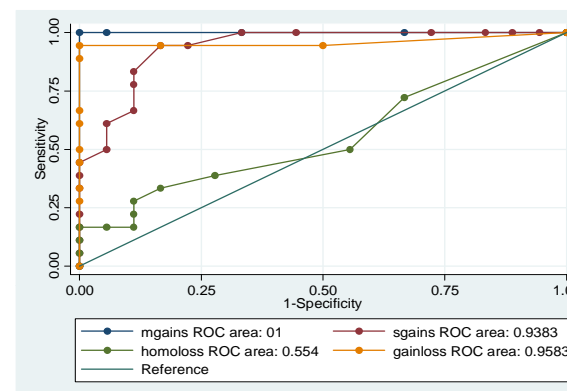
Training Set  
FISH Probe Assay Results

Median % cells (Range)	NSE group (n=18)	EAC group (n=18)	P-value
Multiple probe gains	1 (0 - 3)	51 (11 - 76)	< 0.0001
Single probe gains	6 (2 - 26)	24 (9 - 61)	< 0.0001
Homozygous 9p21 loss	2 (0 - 8)	2 (0 - 73)	0.57
Any gain plus any 9p21 loss	1 (0 - 2)	7 (0 - 70)	< 0.0001

Distribution of FISH Results in Training Set



FISH ROC Analysis in Training Set



- The following thresholds for detection of EAC were established to achieve 90% specificity in the training set:
  - Multiple probe gains ≥4%**
  - Single probe gains ≥16%**
  - Homozygous 9p21 loss ≥9%**
  - Any probe gains with any 9p21 loss ≥4%**

Validation Set FISH Probe Assay Results

Median % cells (Range)	NDBE group (n=33)	EAC group (n=21)	P-value
Multiple probe gains	1 (0 - 25)	34 (2 - 94)	<0.0001
Single probe gains	4 (0 - 51)	15 (5 - 47)	<0.0001
Homozygous 9p21 loss	0 (0 - 10)	1 (0 - 21)	0.01
Any gain plus any 9p21 loss	0 (0 - 9)	6 (1 - 50)	<0.0001

FISH Probe Performance Characteristics in Validation Set

	Area under ROC curve	Sensitivity	Specificity
Multiple probe gains	0.93	95%	91%
Single probe gains	0.74	57%	91%
Homozygous 9p21 loss	0.56	14%	97%
Any gain plus any 9p21 loss	0.78	62%	94%
<b>Any positive test</b>	<b>0.90</b>	<b>95%</b>	<b>85%</b>

## Conclusions

- DNA abnormalities detected by FISH assay were more prevalent among specimens with EAC rather than NDBE
- Neoplasia was detected with high sensitivity and specificity, particularly by assessing for multiple probe gains
- Future study of this assay for the detection of intermediate grades of Barrett's esophagus is warranted

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