

<b>Patient Name:</b> John Doe	<b>Requesting Physician:</b> David A. Smith, M.D.	<b>Collected:</b> 03/13/2009
<b>Sex:</b> Male	<b>Client/Group Facility:</b> GI Associates, PA	<b>Received:</b> 03/20/2009
<b>Date of Birth (Age):</b> 10/10/1958 (50 years)	<b>Additional Recipient:</b> Mark Howard, M.D.	<b>Reported:</b> 03/23/2009
<b>SSN:</b> 212-22-2234	<b>Submitting Pathologist:</b> Tim Jackson, M.D.	<b>Case #:</b> C09-123445
<b>Patient ID:</b> 23445921	<b>Specimen ID:</b> 232323233	<b>Req #:</b>

## Barrett's BioStrat™ Assay

### CLINICAL HISTORY

History of Barrett's Esophagus.

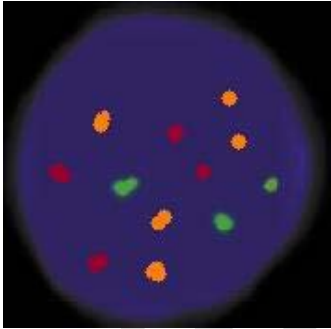

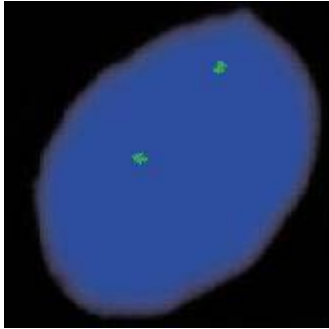
### RESULTS

**POSITIVE.** Enumeration of DNA targets revealed greater than or equal to 4 cells showing gains for two or more loci (8q24, 20q13, and 7p12).

### INTERPRETATION

**High-Risk FISH Results.** Enumeration of 100 consecutive, non-inflammatory cells revealed gains for multiple loci in 40 (40%) of cells. These findings are most consistent with active and/or potential high-grade dysplasia (HGD) or esophageal adenocarcinoma (EA). Clinical and histological correlation is suggested.

### IMAGES FROM ARIOL® SYSTEM

C-MYC (8q24), ZNF217 (20q13), and EGFR (7p12)	Chromosome 17 and HER-2/neu (17q12)	Chromosome 9 and P16 (9p21)
		
Abnormal result observed in an interphase cell obtained from a sample showing three copies of C-MYC (green), five copies of ZNF217 (orange), and four copies of EGFR (red) after the Barrett's BioStrat™ Assay hybridization.	Abnormal result observed in an interphase cell obtained from a sample showing two copies of chromosome 17 (green) and four copies of HER-2/neu (orange) after the Barrett's BioStrat™ Assay hybridization.	Abnormal result observed in an interphase cell obtained from a sample showing two copies of chromosome 9 (green) and absence of both copies of P16 (orange) after the Barrett's BioStrat™ Assay hybridization.

### REFERENCE RANGE

#### POSITIVE

- 1) ≥4 cells showing gains for 2 or more loci (8q24, 20q13, and 7p12); or
- 2) ≥5% cells showing gains for a single locus (8q24, 20q13, 7p12 or 17q12); or
- 3) ≥6% cells with homozygous loss of the 9p21 locus; or
- 4) ≥11% cells with any combination of homozygous and heterozygous 9p21 loss

#### UNINFORMATIVE

Insufficient specimen quality, inadequate slide preparation, or fewer than 100 evaluable cells.

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### ENUMERATION SUMMARY

<b>C-MYC (8q24), ZNF217 (20q13), and EGFR (7p12) Loci</b>		Total number of cells analyzed: 100	
<b>Gains for Multiple Loci (8q24, 20q13, and 7p12):</b>	40 (40%)	<b>Gains for a single Locus (8q24, 20q13 or 7p12):</b>	7 (7%)

#### Criteria for categorization of 8q24, 20q13 and 7p12 loci.

- Gains for multiple loci:** cells that show gains ( $\geq 3$  copies) for two or more of the 8q24, 20q13, and 7p12 loci.
- Gains for a single locus:** cells that show gains ( $\geq 3$  copies) for one of the 8q24, 20q13 or 7p12 loci.

<b>Chromosome 17 and HER-2/neu (17q12) Locus</b>		Total number of cells analyzed: 100	
<b>Locus Gain (17q12):</b>	5 (5%)	<b>17q12 to Chromosome 17 ratio:</b>	1.10

#### Criteria for categorization of chromosome 17 and HER-2/neu (17q12) locus.

- Locus gain:** cells that show gains ( $\geq 3$  copies) for the 17q12 locus.
- 17q12 to chromosome 17 ratio:** ratio of HER-2/neu gene to chromosome 17 copy number.

<b>Chromosome 9 and P16 (9p21) Locus</b>		Total number of cells analyzed: 100	
<b>Homozygous Locus Loss (9p21):</b>	5 (5%)	<b>9p21 to Chromosome 9 ratio:</b>	0.92
<b>Heterozygous Locus Loss (9p21):</b>	2 (2%)		

#### Criteria for categorization of chromosome 9 and 9p21 locus.

- Locus loss:** cells can show a homozygous or heterozygous loss of 9p21 signals.
  - Homozygous loss:** absence of both 9p21 signals.
  - Heterozygous loss:** presence of only one 9p21 signal.
- 9p21 to chromosome 9 ratio:** ratio of P16 gene to chromosome 9 copy number.

### INTENDED USE

The Barrett's BioStrat™ Assay is a fluorescence *in situ* hybridization (FISH) assay that was developed for the detection of cytogenetic evidence of Barrett's associated neoplasia in esophageal cytology brush specimens of patients undergoing surveillance endoscopy for Barrett's esophagus. It consists of fluorescently labeled DNA probes to chromosomes 9, 17, and 8q24, 20q13.2, 17q11.2-12, 7p12, and 9p21 loci containing the C-MYC, ZNF217, HER-2/neu, EGFR, and P16 genes, respectively.

When hybridized and visualized, these probes provide quantitative information on DNA alterations relating to the detection of active and/or potential Barrett's associated neoplasia and the differentiation of low-grade dysplasia from high-grade dysplasia/adenocarcinoma. With the assay result and taking into account other standard diagnostic procedures, the physician and patient can make more informed decisions regarding the surveillance and management of Barrett's esophagus.

### BACKGROUND

Barrett's esophagus is a condition in which the normal mucosa of the distal esophagus is replaced by an intestinal type columnar epithelium. Patients with this condition are at an increased risk for developing adenocarcinoma of the esophagus compared to the general population.<sup>1-4</sup> The evolution to esophageal adenocarcinoma (EA) from Barrett's esophagus (BE) is generally accepted to be a multistep progression as follows: 1) intestinal metaplasia (IM) of the normal stratified squamous epithelium, 2) low-grade dysplasia (LGD), 3) high-grade dysplasia (HGD) and 4) EA.

Chromosomal aberrations are a hallmark of malignancy and also present in Barrett's associated neoplasia. FISH is a technique that uses fluorescently labeled DNA probes to detect chromosomal alterations in cells and is thus increasingly used as an adjunct method in the diagnosis and surveillance of malignancies. Recently, studies have demonstrated the ability of FISH to detect chromosomal alterations in cells collected from endoscopic brushings and have identified probe sets useful for the detection of dysplasia and esophageal adenocarcinoma, as well as differentiate low-grade dysplasia from high-grade dysplasia/adenocarcinoma in patients with Barrett's esophagus.<sup>5-7</sup>

### METHODOLOGY

**Slide Preparation and Pretreatment.** Esophageal cytology brush specimens for Barrett's BioStrat™ analysis are prepared using the liquid-based cytology technique (ThinPrep®, Cytoc, Boxborough, MA). The prepared specimen slides are protease digested, fixed in formaldehyde, washed, and dehydrated.

**FISH Assay.** The FISH assay is performed using three separate, multicolor interphase FISH probe sets. The C-MYC/ZNF217/EGFR probe

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set is a mixture of three locus-specific indicator (LSI) probes; C-MYC labeled with SpectrumGreen, ZNF217 labeled with SpectrumOrange, and EGFR labeled with SpectrumOrange that hybridize to the 8q24 region of chromosome 8, the 20q13.2 region of chromosome 20, and the 7p12 region of chromosome 7, respectively. The C-MYC, ZNF217, and EGFR LSI probes are used to determine gene copy number or amplification.

The HER-2/CEP 17 probe set is a mixture of the LSI HER-2 probe labeled with SpectrumOrange and the chromosome enumeration probe (CEP) 17 labeled with SpectrumGreen. The LSI HER-2 SpectrumOrange probe hybridizes to the 17q11.2-12 region which has been found to contain the HER-2/neu gene. The CEP 17 SpectrumGreen probe hybridizes to alpha satellite sequences specific to chromosome 17. The HER-2/CEP 17 probe set is designed to detect amplifications of the HER-2/neu gene. Inclusion of the CEP 17 probe allows for the relative copy number of the HER-2/neu gene to be determined.

The P16/CEP 9 probe set is a mixture of the LSI P16 probe labeled with SpectrumOrange and the CEP 9 probe labeled with SpectrumGreen. The LSI P16 SpectrumOrange probe hybridizes to the 9p21 region which has been found to contain the P16 tumor suppressor gene. The CEP 9 SpectrumGreen probe hybridizes to alpha satellite sequences specific to chromosome 9. The P16/CEP 9 probe set is designed to detect 9p21 deletions.

**Enumeration of FISH Signals.** Determination of results is conducted by enumeration of CEP 9, 17 and LSI C-MYC, ZNF217, EGFR, HER-2, and P16 signals through automated interphase FISH analysis using the Ariol® digital image analysis system (Genetix, New Milton, UK) equipped with appropriate excitation and emission filters allowing visualization of the orange, green, and red fluorescent signals. Specimens hybridized with the Barrett's BioStrat™ multi-color probe mixture sets will exhibit signals indicative of the copy number of chromosomes 9, 17 and the 8q24, 20q13, 7p12, 17q12 and 9p21 regions.

**Interpretation of Results.** A minimum of 100 consecutive, non-inflammatory cells per FISH probe set are analyzed. The signal distribution for cells showing either single or multiple gains of the CEP 9, 17 and 8q24, 20q13, 7p12 or 17q12 loci or a loss of the 9p21 locus is recorded. The total number of chromosomally abnormal cells, i.e., cells with gains of 8q24, 20q13, 7p12 or 17q12 or loss of the 9p21 locus is determined and the results are reported as positive or negative along with semi-quantitative findings including pattern of locus abnormality (i.e., gains of 8q24, 20q13, 7p12, and 17q12 and homozygous/heterozygous 9p21 loss) and percent abnormal cells.

### CLINICAL CONSIDERATIONS

**Quantitative Molecular Cytology.** Numerous studies have identified common genetic alterations associated with low-grade dysplasia (LGD), high-grade dysplasia (HGD), and esophageal adenocarcinoma (EA) in patients with Barrett's esophagus. Genes or genetic loci that have been found to be frequently altered include: 3p21, 5p15, 5q21-22, EGFR (7p12), 7q36.1, C-MYC (8q24.12-13), P16 (9p21), P53 (17p13.1), HER-2/NEU (17q11.2-12), 20q13.2, and the Y chromosome.<sup>8-10</sup> Recently, Brankley et al. identified a set of FISH probes consisting of locus-specific probes to 8q24.12-13, 9p21, 17q11.2-12, and 20q13.2 with the potential to provide high sensitivity and specificity for the detection of Barrett's associated neoplasia and to differentiate high-grade dysplasia/adenocarcinoma from low-grade dysplasia.<sup>5</sup> This probe set was found to have a sensitivity and specificity, respectively, of 70% and 89% for low-grade dysplasia, 84% and 93% for high-grade dysplasia, and 94% and 93% for esophageal adenocarcinoma.

**Anticipatory Positive Results.** The FISH assay is quite sensitive, and it is not uncommon for the assay to be positive for a patient with a negative pathology result (i.e., 'false-positive' FISH results). A significant proportion of these 'false-positive' FISH results are not believed to be true false-positive results but rather believed to represent cases in which FISH has detected an abnormality that was not detected by the 'gold standard' (i.e., biopsy). This phenomenon has previously been observed when using FISH to detect recurrent bladder cancer in patients being monitored for tumor recurrence. Long-term follow-up of these patients has shown that a high proportion of these patients with apparent false-positive FISH results eventually develop biopsy proven tumor. For this reason, false-positive results are sometimes referred to as 'anticipatory positive' FISH results since they frequently represent cases in which tumor has been detected before it can be identified by other means.

In a recent study, Fritcher et al. found that eighty-five percent of patients with a FISH result showing multiple loci gains had HGD or EA at the time of the initial biopsy, and the remaining 15% progressed to HGD or EA within 6 months.<sup>11</sup> For patients with 9p21 loss, 43% had HGD or EA on the initial biopsy and another 24% progressed within 4 months. In comparison, only 13% of patients with a negative FISH result had HGD/EA on the original biopsy, with an additional 18% progressing to HGD/EA after 22 months. This analysis indicates that regardless of the original biopsy diagnosis, patients with a FISH result showing multiple loci gains are at highest risk for progression to HGD/EA, followed by patients with 9p21 loss and single locus gain.

**Risk Stratification and Surveillance Strategies.** FISH performed on esophageal brushing specimens seems to be a promising method with which to screen patients with BE for cytogenetic abnormalities associated with dysplasia and/or malignancy. Analysis of ongoing data shows that patients with multiple loci gains detected in a brushing specimen progress to HGD/EA significantly earlier than patients with other FISH abnormalities. The finding of 9p21 loss or gain of a single locus appears to be associated with a risk for progression that is less than patients with a result showing multiple loci gains but significantly greater than those with a negative FISH result. Because of the less invasive nature of cell collection for this assay compared with the traditional biopsy protocol and the fact that test results stratify patients for risk of progression to severe lesions, FISH is a promising modality to augment and refine current surveillance methods.

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If you have any questions on this report please do not hesitate to contact the BioVantra client support center at (866) 627-8221.

**Peter A. Tsivis, M.D.**  
Pathologist Electronic Signature

**03/23/2009**  
Date

This test was developed and its performance characteristics determined by BioVantra, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). However, the FDA has determined that such approval is not necessary. This test is permitted for clinical purposes and should not be regarded as purely investigational or for research. BioVantra is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

Photomicrographs are a symbolic representation of the key findings of the Barrett's BioStrat™ Assay report. The photomicrographs are not intended to replace a complete review and reading of the final diagnostic report provided.