Endoscopic mucosal tissue sampling

This is one of a series of statements discussing the use of GI endoscopy in common clinical situations. The Standards of Practice Committee of the American Society for Gastrointestinal Endoscopy (ASGE) prepared this text. In preparing this guideline, a search of the medical literature was performed by using PubMed. Additional references were obtained from the bibliographies of the identified articles and from recommendations of expert consultants. When few or no data exist from well-designed prospective trials, emphasis is given to results from large series and reports from recognized experts. Guidelines for appropriate use of endoscopy are based on a critical review of the available data and expert consensus at the time that the guidelines are drafted. Further controlled clinical studies may be needed to clarify aspects of this guideline. This guideline may be revised as necessary to account for changes in technology, new data, or other aspects of clinical practice. The recommendations are based on reviewed studies and are graded on the strength of the supporting evidence (Table 1). The strength of individual recommendations is based on both the aggregate evidence quality and an assessment of the anticipated benefits and harms. Weaker recommendations are indicated by phrases such as “We suggest...” whereas stronger recommendations are typically stated as “We recommend....” These statements are included in Table 2, rather than as specific statements, as in other Standards of Practice documents.

This guideline is intended to be an educational device to provide information that may assist endoscopists in providing care to patients. This guideline is not a rule and should not be construed as establishing a legal standard of care or as encouraging, advocating, requiring, or discouraging any particular treatment. Clinical decisions in any particular case involve a complex analysis of the patient’s condition and available courses of action. Therefore, clinical considerations may lead an endoscopist to take a course of action that varies from these guidelines.

GI endoscopy and tissue acquisition are fundamental to the diagnosis and management of diseases of the digestive system. The proper collection of tissue specimens is required for accurate pathologic diagnosis. Communication between endoscopist and pathology colleagues facilitates effective tissue collection and analysis. Indications, equipment, and techniques for endoscopic tissue sampling have been reviewed in other ASGE publications. This document serves as a complement to previous reports, with a focus on mucosal tissue acquisition. Unless otherwise specified, all forceps biopsies refer to standard sized forceps. Table 2 is a compilation of tissue sampling recommendations and suggestions discussed in this as well as other ASGE documents.

SPECIMEN HANDLING IN THE ENDOSCOPY UNIT

Immediately after sampling, a mucosal specimen should be gently submerged into a jar containing the appropriate fixation fluid to minimize tissue desiccation and preserve tissue architecture. Specimens obtained for specific tests, such as tissue culture, molecular tests, and electron microscopy, should be handled according to the guidelines in place at the home institution. Specimens for culture should preferably be obtained first.

The fixative used for most GI mucosal biopsies is 10% buffered formalin. Formalin provides excellent tissue fixation and allows staining by routine histologic methods (eg, hematoxylin and eosin, immunohistochemistry) with optimal results. Many molecular tests also can be performed on formalin-fixed tissue. Specimens that require placement in nonfixation fluids should be handled with a tissue-capture device that has not been previously placed in fixative. It is suggested that specimen collection devices used to sample malignant tissue not be subsequently used to obtain samples from suspected normal tissue to avoid the theoretical possibility of malignant contamination.

Most GI biopsies do not require specimen orientation for definitive pathologic interpretation. However, properly oriented specimens are always preferred by pathologists and can provide important and, in some cases, essential pathologic information. For instance, basal cell hyperplasia in esophageal biopsy samples in patients with GERD or thickness of the subepithelial collagen layer in patients with suspected microscopic colitis can be interpreted with greater ease and accuracy in properly oriented biopsy specimens.

Communication with pathology staff is encouraged to understand institutional standards regarding the specifics of mucosal specimen collection and submission. Discussion with the local pathologist can clarify the recommended
size of specimen jar, the amount of solution needed for sample immersion, and the maximum number of samples placed in a specimen container. Guidelines for orientation of polypectomy specimens and desired information to be included on specimen labels and GI pathology requisitions may also be helpful to clarify with local pathology colleagues.

**ESOPHAGUS**

**GERD**

There is no established biopsy protocol for GERD in the absence of recognized Barrett’s metaplasia or eosinophilic esophagitis (EoE). Biopsies of normal-appearing distal esophageal mucosa in patients with GERD symptoms may reveal nonspecific changes, known as minimal change esophagitis, defined as papillary elongation, basal cell hyperplasia, and dilation of intercellular space. The clinical implications of histologic esophageal mucosal abnormalities in the absence of endoscopically visible changes are uncertain, and biopsy of endoscopically normal mucosa in GERD, when other diagnoses are not suspected, is not recommended.12-17

**Eosinophilic esophagitis**

Variable diagnostic criteria and a patchy microscopic and macroscopic distribution have made standardization of a biopsy protocol for EoE difficult.18,19 An interdisciplinary expert panel of 33 physicians recommended taking 2 to 4 biopsy samples each from the proximal and distal esophagus, even if the esophageal mucosa appears normal. Biopsy samples should also be taken of the gastric antrum and duodenum when there is a suspicion of eosinophilic gastroenteritis.20 Biopsy samples should not be placed in Bouin’s preservative, which can lead to a reduced ability to identify eosinophils.21 Differentiating the pathologic changes of EoE from those of GERD can sometimes prove challenging.

**Infectious esophagitis**

**Cytomegalovirus and herpes simplex virus.** Cytomegalovirus (CMV) infects mesenchymal and columnar cells and presents macroscopically as ulcerative lesions. Biopsy for CMV should be concentrated at the ulcer base to optimize sampling and diagnostic accuracy.22 In a study of HIV-infected patients with esophageal ulcers, 3 forceps biopsy samples from the ulcer base were diagnostic in 80% of patients with CMV esophagitis, with a maximum of 10 biopsy samples needed to confirm the diagnosis in the remaining patients. The biopsy samples were examined by using standard histopathologic methods, with in situ hybridization or immunohistochemical stains as needed.23 Qualitative CMV polymerase chain reaction of biopsy samples is more sensitive than standard histopathology, but likely detects latent as well as clinical disease.24 Data are inconsistent regarding the benefit of viral culture in the evaluation of CMV esophagitis.25,26

Herpes simplex virus infects squamous epithelial cells, present at the lateral margin of ulcers and erosions, so mucosal biopsy samples of the ulcer margin have the highest diagnostic yield.27 Viral culture and polymerase chain reaction can aid in diagnosing herpes simplex virus esophagitis.27-29

**Candida.** There are limited data on the optimal diagnostic technique for esophageal candidiasis. Cytologic brushing may be more sensitive than histology for diagnosis.25,30

**STOMACH**

**Helicobacter pylori gastritis.** The diagnostic test used for *H pylori* detection depends on the clinical situation, cost considerations, and local expertise. Endoscopic tests include tissue urease activity, histologic examination, and microbial culture. The sensitivity of these tests may be decreased by proton pump inhibitors, bismuth compounds, antibiotics, and acute GI bleeding.31,32 In situations in which test sensitivity is reduced, a negative urease test result should be confirmed with a different test for *H pylori* infection, with histology being a convenient alternative.33 *H pylori* culture allows identification of the bacterial strain and antimicrobial resistance patterns, but is difficult to obtain and performed at only a few centers.

There are limited data on the optimal biopsy protocol for the diagnosis of *H pylori*. Specifics of biopsy protocols are listed in Table 2.34,35 El-Zimaity et al reported that
Gastric epithelial polyps include fundic gland polyps, hyperplastic polyps, and adenomas. Gastric polyps are often incidentally detected on endoscopy. Polyp histology cannot be reliably distinguished by endoscopic appearance. Endoscopic forceps biopsy is inadequate to rule out dysplasia and carcinoma for polyps larger than 0.5 cm to 1 cm.\(^\text{38-40}\) Fundic gland polyps that develop sporadically or in association with long-term proton pump inhibitor use have very low to no malignant potential.\(^\text{41}\) It should be realized, however, that dysplasia may be found in fundic gland polyps associated with familial adenomatous polyposis.\(^\text{12}\)

Dysplastic elements may be present in as many as 20% of hyperplastic polyps.\(^\text{43}\) Adenomatous gastric polyps also have malignant potential. Hyperplastic and adenomatous polyps may occur in the presence of *H pylori* infection and EMAG, and sampling of these entities should be performed. When hyperplastic and adenomatous polyps are identified or suspected based on endoscopic appearance, biopsy samples should also be taken from the surrounding nonpolypoid mucosa to exclude dysplasia arising from a background of metaplastic atrophic gastritis.

**SMALL INTESTINE**

**Celiac disease**

Recommendations regarding mucosal tissue acquisition for the diagnosis of celiac disease have been based on expert opinion as well as emerging literature. Multiple biopsy samples taken from multiple sites are thought to help to avoid inadequate sampling caused by the patchy nature of the disease and biopsy crush artifact and allow proper specimen orientation.\(^\text{44-46}\) Celiac disease may be localized to the duodenal bulb.\(^\text{47-50}\) In patients with suspected celiac disease, we recommend 4 to 6 biopsy samples be obtained with standard forceps from the duodenal bulb and more distal duodenum. Although endoscopically abnormal mucosa should be preferentially targeted for sampling, it is important to realize that histologic disease may underlie normal-appearing mucosa.

Historically, it was thought that well-oriented biopsy samples made it easier for pathologists to identify the cardinal features of celiac disease. Recent data, however, suggest that patients with celiac disease may only show increased intraepithelial lymphocytosis (as opposed to villous atrophy) as the key diagnostic finding.\(^\text{72}\) Increased intraepithelial lymphocytosis is a feature that does not rely heavily on specimen orientation.

**COLON**

**Microscopic colitis**

No consensus exists for the optimal method of mucosal sampling in suspected microscopic colitis. The potential patchy distribution of microscopic colitis sometimes requires biopsy of the right and transverse colon, in addition to the left side of the colon, for diagnosis.\(^\text{21,51-53}\) A reasonable initial strategy may be to proceed with either flexible sigmoidoscopy or colonoscopy, with biopsy samples as detailed in Table 2. In situations in which flexible sigmoidoscopy is nondiagnostic, but clinical suspicion for microscopic colitis remains high, colonoscopy with additional mucosal sampling from the proximal colon should be considered. No formal comparative effectiveness analysis exists comparing and evaluating the 2 strategies.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Tissue sampling</th>
<th>Strength</th>
<th>Evidence</th>
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</thead>
<tbody>
<tr>
<td><strong>Esophagus</strong></td>
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<tr>
<td>GERD</td>
<td>Targeted biopsies of irregular mucosa, if clinically warranted</td>
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<td>4 4</td>
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<tr>
<td>BE*</td>
<td>Surveillance of nondysplastic BE: 4-quadrant biopsies every 2 cm with large-capacity forceps for length of Barrett’s mucosa</td>
<td>S</td>
<td>4 4</td>
</tr>
<tr>
<td></td>
<td>Surveillance of BE with LGD: 4-quadrant biopsies every 1-2 cm with large-capacity forceps for length of Barrett’s mucosa</td>
<td>R</td>
<td>4 4</td>
</tr>
<tr>
<td></td>
<td>Surveillance of BE with HGD: 4-quadrant biopsies every 1 cm with large-capacity forceps for length of Barrett’s mucosa</td>
<td>R</td>
<td>4 4</td>
</tr>
<tr>
<td>EoE*</td>
<td>2-4 biopsies from proximal esophagus 2-4 biopsies from distal esophagus Biopsies of gastric antrum and duodenum when EG suspected Biopsies should not be placed in Bouin’s preservative</td>
<td>S</td>
<td>4 4</td>
</tr>
<tr>
<td>Infectious esophagitis</td>
<td>Viral esophagitis Multiple biopsies from margin and base of visualized ulcers Specimens should be sent for standard histology, IHC, and possibly viral culture and PCR</td>
<td>S</td>
<td>4 4</td>
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<tr>
<td></td>
<td>Candidal esophagitis Multiple biopsies of affected area Cytologic brushing complementary to biopsy</td>
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<tr>
<td><strong>Stomach</strong></td>
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<td><em>H pylori</em> infection*</td>
<td>Urease test 1-2 biopsies: 5 cm proximal to the pylorus on the lesser curvature near the angularis or on the greater curve opposite the angularis A negative urease test result should be confirmed with further testing for <em>H pylori</em></td>
<td>S</td>
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<td></td>
<td>Histologic diagnosis 2 approaches: 3 biopsies: 1 from the angular corpus-antrum junction, 1 from the greater curvature of the corpus, 1 from the greater curvature of the antrum or Updated Sydney Protocol 5 biopsies: 1 from antrum the 2-3 cm from the pylorus lesser curvature, 1 from the antrum 2-3 cm from the pylorus greater curvature, 1 from the corpus 8 cm from the cardia lesser curvature, 1 from the corpus 8 cm from the cardia greater curvature, 1 from the angularis</td>
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<tr>
<td></td>
<td>If <em>H pylori</em> positive, perform biopsy protocol for EMAG (below)</td>
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<td>4 4</td>
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<tr>
<td>Environmental metaplastic atrophic gastritis*</td>
<td>Best data with 7-12 biopsies: 4-quadrant biopsies from antrum (2-3 cm proximal to pylorus), 2 from the angularis, 4 from the mid corpus (2 lesser curvature, 2 greater curvature), 2 from the cardia</td>
<td>S</td>
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<td>EMAG protocol should be sufficient for <em>H pylori</em> histologic diagnosis</td>
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<tr>
<td>Autoimmune metaplastic atrophic gastritis*</td>
<td>Individualize approach. Biopsies directed at ulcers, nodules, polyps, masses to rule out neoplasia</td>
<td>S</td>
<td>4 4</td>
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<tr>
<td>Disease</td>
<td>Tissue sampling</td>
<td>Strength</td>
<td>Evidence</td>
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<tr>
<td>Gastric polyps*</td>
<td>Solitary polyps Sample (biopsy or polypectomy).</td>
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<td></td>
<td>Further management determined by histology: Fundic gland polyp: $\geq 1 \text{ cm}$ should undergo polypectomy</td>
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<tr>
<td></td>
<td>Hyperplastic polyp: $\geq 0.5 \text{ cm}$ should undergo polypectomy</td>
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<tr>
<td></td>
<td>Adenomatous polyp: all should undergo polypectomy</td>
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<tr>
<td></td>
<td>Multiple polyps Largest polyp removed with polypectomy</td>
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<td></td>
<td>Representative sampling of smaller polyps</td>
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<td></td>
<td>Further management dependent on histology</td>
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<tr>
<td>Peptic ulcer disease</td>
<td>Multiple biopsies from the base and edges of a gastric ulcer when malignancy</td>
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<td>is suspected or suggested by endoscopic appearance. Cytology may be complementary</td>
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<td>4</td>
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<tr>
<td></td>
<td>Perform biopsy protocol for <em>H. pylori</em> as above</td>
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<td>4</td>
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</tbody>
</table>

**Small intestine**

| Celiac disease, tissue sampling instructions, strength, and evidence grades | 4-6 biopsies in total from duodenal bulb and distal duodenum | $        | 4        |

**Colon**

| Microscopic colitis       | 2 approaches:                                              | $        | 4        |
|                         | 1. FS: $\geq 2$ biopsies from the transverse colon (if possible) | $        | 4        |
|                         | $\geq 2$ biopsies from the sigmoid colon                    | $        | 4        |
|                         | $\geq 2$ biopsies from the descending colon                  | $        | 4        |
|                         | or                                                           | $        | 4        |
|                         | 2. Colonoscopy: $\geq 2$ biopsies from the right colon       | $        | 4        |
|                         | $\geq 2$ biopsies from the transverse colon                  | $        | 4        |
|                         | $\geq 2$ biopsies from the descending colon                  | $        | 4        |
|                         | $\geq 2$ biopsies from the sigmoid colon                     | $        | 4        |
|                         | A nondiagnostic FS should be followed by colonoscopy with biopsy, pending clinical suspicion | $        | 4        |

**Inflammatory bowel disease*\**

| Initial diagnosis        | $\geq 2$ biopsies from at least 5 sites, including the ileum and rectum | $        | 4        |
|                         | If EGD performed for suspected IBD: $\geq 2$ biopsies from the esophagus | $        | 4        |
|                         | $\geq 2$ biopsies from the stomach                              | $        | 4        |
|                         | $\geq 2$ biopsies from the duodenum                            | $        | 4        |
|                         | Surveillance for dysplasia: all endoscopically visible lesions should undergo biopsy and/or be removed | R       | 4        |
|                         | 2 approaches:                                                  | R       | 4        |
|                         | 1. Chromoendoscopy with pancolonial dye spraying and targeted biopsies. Also, biopsies from each colonic segment to assess inflammation. | R       | 4        |
Inflammatory bowel disease

Biopsy protocols to diagnose, stage, and survey patients with inflammatory bowel disease (IBD) are based on expert consensus, case series, case-control studies, population-based cohort studies, prospective studies, and, in the case of optimal technique for surveillance colonoscopy, controlled trials. In patients undergoing ileocolonoscopy for suspected IBD, at least 2 biopsy samples should be taken from 5 sites, including the ileum and rectum, during the initial endoscopic evaluation. Specimens should be taken of both diseased and adjacent normal-appearing mucosa. The biopsy specimens from different locations should be separately labeled to allow staging of the extent and severity of disease. Higher detection rates for granulomas can be achieved when biopsy specimens are taken from the edge of ulcers and aphthous erosions. If an upper endoscopy (EGD) is performed for clinically suspected upper GI tract IBD, at least 2 biopsy samples should be taken from the esophagus, stomach, and duodenum. Surveillance for colorectal cancer is recommended for patients with long-standing IBD. Recent studies indicate that most dysplasia is endoscopically visible.

<table>
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<tr>
<th>Disease</th>
<th>Tissue sampling</th>
<th>Strength</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Pancolitis: 4-quadrant biopsies every 10 cm from the cecum to the rectum, for a minimum total 33 biopsy samples</td>
<td>S</td>
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<tr>
<td>Nonpancolitis: 4-quadrant biopsies every 10 cm limited to greatest extent of endoscopic or histologic involvement documented by any colonoscopy</td>
<td>S</td>
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<tr>
<td>Suspected pouchitis</td>
<td>Multiple biopsies from the pouch and afferent limb</td>
<td>S</td>
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</table>

**Miscellaneous**

- **Acute graft-versus-host disease**
  - 2 approaches:
    - 1. FS
      - ≥4 standard forceps biopsies from the rectosigmoid; ≥4 standard forceps biopsies from the left colon
      - If FS nondiagnostic, proceed to EGD with ≥4 standard forceps biopsies each, from the gastric antrum, gastric body, and duodenum.
      - Distal esophageal biopsies may be considered.
    - 2. Ileocolonoscopy
      - ≥4 standard forceps biopsies from the terminal ileum
      - ≥4 standard forceps biopsies from the right colon
      - ≥4 standard forceps biopsies from the transverse colon
      - ≥4 standard forceps biopsies from the left colon
      - ≥4 standard forceps biopsies from the rectosigmoid

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S, "We suggest..."; R, "We recommend..."; BE, Barrett’s esophagus; LGD, low-grade dysplasia; HGD, high-grade dysplasia; EoE, eosinophilic esophagitis; EG, eosinophilic gastroenteritis; IHC, immunohistochemistry; PCR, polymerase chain reaction; EMAG, environmental metaplastic atrophic gastritis; FS, flexible sigmoidoscopy; IBD, inflammatory bowel disease; PPI, proton pump inhibitor.

Recommendations in Table 2 are as specific as published literature allows. Consensus recommendations often do not exist. Comparative effectiveness and cost-effectiveness studies were not available to guide tissue sampling suggestions. As a general principle of tissue acquisition, we suggest that specimen collection devices used to sample malignant tissue not be subsequently used on normal tissue to minimize the theoretical possibility of malignant contamination. For all conditions, in addition to the protocol listed, targeted biopsy samples should also be taken from any mucosal irregularities and sent in their own container for pathologic examination.

*Specimens taken from a given geographic location should be submitted to pathology in their own container.

| Recommendation is for a sporadic single fundic gland polyp that is not associated with familial adenomatous polyposis. Although sporadic fundic gland polyps have low to no malignant potential, expert opinion has been to remove fundic gland polyps larger than 1 cm. Withdrawal of a proton pump inhibitor may be considered in patients with fundic gland polyps larger than 1 cm.

| If *H pylori* positive, treatment has been associated with regression of hyperplastic polyps.

| Consideration should be given to sampling every 5 cm in the distal colon.

| Although no hemorrhagic complications with biopsy were noted in the prospective literature, they have been reported in a retrospective study. Clinical judgment should be used to reduce number of biopsies if there is thought to be a significant bleeding risk.

| Alternatively, pending clinical scenario, flexible sigmoidoscopy/EGD may be performed consecutively during a single endoscopic session.
colonoscopy, targeted biopsies of all visible abnormalities should be performed. To optimize detection of dysplastic changes when using standard white light endoscopy, chromoendoscopy by using methylene blue or indigo carmine with targeted biopsies is recommended when expertise in this technique is available, with additional biopsy samples of all colonic segments to stage the extent of inflammation. A meta-analysis of prospective studies comparing chromoendoscopy with standard white light endoscopy demonstrated a pooled incremental yield of chromoendoscopy over standard white light endoscopy for the detection of any grade of dysplasia on a per-patient basis of 7% (95% CI, 3.2-11.3), with a number needed to treat of 14 patients to detect 1 additional patient with dysplasia or cancer. No study has compared the yield of high-definition colonoscopy with high-definition chromoendoscopy. When chromoendoscopy is not available, especially if there is extensive active disease, significant pseudopolyposis, or poor preparation precluding complete evaluation, random mucosal sampling with targeted biopsies of any suspicious-appearing lesions remains a reasonable alternative. In patients with documented pancolitis, biopsy specimens should be obtained in a 4-quadrant fashion every 10 cm from the cecum to the rectum for a minimum of 33 total random mucosal samples in an attempt to detect dysplastic changes. In patients with less extensive colitis, biopsy specimens can be limited to the greatest extent of endoscopic or histologic involvement documented by any colonoscopy. Because of an increased risk of colorectal cancer in the rectum and sigmoid, sampling every 5 cm in the distal colon should be considered. All lesions that appear endoscopically resectable should be removed in their entirety, and biopsy specimens of the flat mucosa surrounding the resection site should be obtained to ensure that the lateral margins are free of dysplasia. The endoscopically removed lesion and the flat mucosa sample from the surrounding the resection site should be placed in separate containers. In patients who have undergone total proctocolectomy with ileal pouch–anal anastomosis who have symptoms consistent with pouchitis, endoscopy with biopsy is indicated. Both the pouch and the afferent small-bowel limb should be carefully evaluated. Any abnormalities of the afferent small bowel should undergo biopsy to evaluate for the possibility of Crohn’s disease.

**REFERENCES**


**DISCLOSURE**

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**ABBREVIATIONS**: aGVHD, acute graft-versus-host disease; AMAG, autoimmune atrophic metaplastic gastritis; CMV, cytomegalovirus; EMAG, environmental metaplastic atrophic gastritis; EoE, eosinophilic esophagitis; IBD, inflammatory bowel disease.


62. Clinical practice guidelines for surveillance colonoscopy in adenoma follow-up; following curative resection of colorectal cancer; and for cancer surveillance in inflammatory bowel disease. Cancer Council Australia; Sydney; 2011.


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