

Comparison of 3 Handling Techniques for Endoscopically Obtained Gastric and Duodenal Biopsy Specimens: A Prospective Study in Dogs and Cats

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Background: Limited evidence exists in the literature regarding whether a specific mount is preferable to use for processing endoscopically obtained gastrointestinal biopsy specimens.

Hypothesis/Objectives: To compare 3 methods of handling endoscopically obtained gastrointestinal biopsy specimens from collection to laboratory processing and to determine if any technique produced superior results.

Animals: Twenty-three dogs and cats presented for gastrointestinal signs.

Methods: Prospective study of dogs and cats presented with gastrointestinal signs to a veterinary teaching referral hospital which underwent upper gastrointestinal endoscopy. Biopsy specimens were taken from the stomach and duodenum and submitted to the laboratory using 3 techniques: mounted on a cucumber slice, mounted on a moisturized synthetic foam sponge, and floating free in formalin. The techniques were compared with regard to the specimens' width, orientation, presence of artifacts, and pathologist's confidence in diagnosis.

Results: Twenty-three patients were included, with a total of 528 biopsies collected. Specimens on cucumber slice and on sponge were significantly wider ($P < .001$ and $P = .001$, respectively) compared to those floating free in formalin (mean width of 3.81 versus 3.31 and 2.52 mm, respectively). However, specimens on synthetic sponge had significantly fewer artifacts compared to those on cucumber slice ($P = .05$) and those floating free in formalin ($P = .02$). Confidence in the diagnosis also was superior with the sponge technique over floating free specimens ($P = .002$).

Conclusions and Clinical Importance: The use of mounted gastrointestinal biopsy specimens was superior over the use of specimens floating free in formalin. This technique improved the quality of the specimens and the pathologist's confidence in their histopathologic interpretation.

Key words: Duodenoscopy; Gastroenterology; Histopathology; Mount.

Endoscopy of the gastrointestinal tract is commonly performed in companion animals to assess macroscopic lesions of the mucosa and obtain targeted biopsy specimens. Because these specimens are inevitably of small size, their quality is critical to reach a reliable diagnosis, and various aspects of their collection and processing, including endoscopist experience, have been investigated.^{1–3} Several studies in human and veterinary medicine have compared different types of endoscopic forceps to improve specimen quality. No single model has demonstrated clear superiority over others,

Abbreviations:

WSAVA World Small Animal Veterinary Association

although some differences were noticed inconsistently regarding the size and depth of the biopsy specimens and the presence of artifacts, with larger forceps generally being preferable.^{4–7} Similarly, retrospective studies assessing the quality of endoscopically obtained samples have recommended a minimum number of 6 biopsy specimens per site of interest to achieve an accurate diagnosis.^{2,3} Subsequently, a consensus statement has summarized these data to establish guidelines for the performance of gastrointestinal endoscopy and biopsy in dogs and cats.⁸

Similarly, a degree of variability has been demonstrated among pathologists regarding histopathologic interpretation, leading to diagnostic discrepancies.⁹ In response, the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group defined guidelines for the interpretation of gastrointestinal inflammation in dogs and cats, in an attempt to standardize the results and allow comparison.¹⁰

The importance of careful handling and proper orientation of endoscopically obtained gastrointestinal biopsy specimens has been emphasized both in human and veterinary medicine.^{3,11} Commonly, the forceps are shaken directly in the formalin solution, and the samples remain floating free in the fixative. Some clinicians use a synthetic foam sponge as a mount to stabilize the specimens. The use of cellulose acetate paper and filter paper for proper orientation, identification and easier handling of the biopsy specimens also has been

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described.^{12,13} In humans, the use of thin cucumber slices as a mount was first described in 1970 to improve orientation of cervical biopsies.¹⁴ The technique also has been reported in preparing bladder and vitreoretinal biopsy specimens.^{15,16} The only use of cucumber slices in veterinary medicine has been reported with lung biopsy specimens in calves.¹⁷ Recent veterinary endoscopy textbooks have reported the use of these different techniques, but without critical appraisal.^{18,19}

To the authors' knowledge, no study has been published to compare the different techniques reported in the literature for handling of endoscopically obtained gastrointestinal biopsy specimens from their collection to processing in the laboratory. A preliminary study conducted in our hospital gave promising results with the use of cucumber slices encased in plastic cassettes. Therefore, the objectives of our study were to compare prospectively 3 different techniques for mounting endoscopically obtained gastrointestinal biopsy specimens, and to determine whether the techniques provide reliable diagnostic histopathologic samples and if any 1 technique is superior. The selected techniques were specimens floating free in formalin (commonly used in veterinary hospitals), specimens mounted on a moisturized synthetic foam sponge encased in a fenestrated plastic cassette (as used in histopathology laboratories), and specimens mounted on a thin cucumber slice encased in the same type of cassette.

Materials and Methods

The study design and materials and methods were reviewed and approved beforehand by Alfort Veterinary School Ethical Committee (ComERC, trial registration number 2014-09-12).

Dogs and cats presented to Alfort Veterinary School Teaching Hospital between September 2014 and February 2015 with clinical signs requiring endoscopy of the upper gastrointestinal tract were prospectively included. Relevant history and clinical signs were recorded for all of the patients. Conventional screening including biochemistry, hematology, and abdominal ultrasound examination also was performed for all patients before endoscopy.

Biopsy collection and sample process

All endoscopies were performed by the same experienced practitioner (VF) with the patients under general anesthesia, and using a gastroscope^a with 2.45 mm diameter forceps^b for biopsy. These forceps were chosen because they had provided samples of good quality in recent studies.^{4,7}

The biopsy specimens were systematically taken from 3 different sites in the stomach (fundus, body, and antrum) and from the duodenum, using the "turn-and-suction" technique.¹ Six different biopsies were taken per site, gently removed from the forceps using a 25G needle and processed in 3 different manners. Two specimens were immersed directly into fixative (10% formalin), 2 specimens were gently mounted and orientated on a small synthetic foam sponge previously moistened with saline, and 2 specimens were gently mounted and oriented on a thin, prepared slice of cucumber. Sponges and cucumber slices then were encased in plastic cassettes and immersed in 10% formalin (Fig 1). The cucumber slices were prepared beforehand as described elsewhere.^{14,18} Briefly, the cucumber was cut in length (excluding seeds) into thin slices 2-mm thick using a kitchen mandolin; the

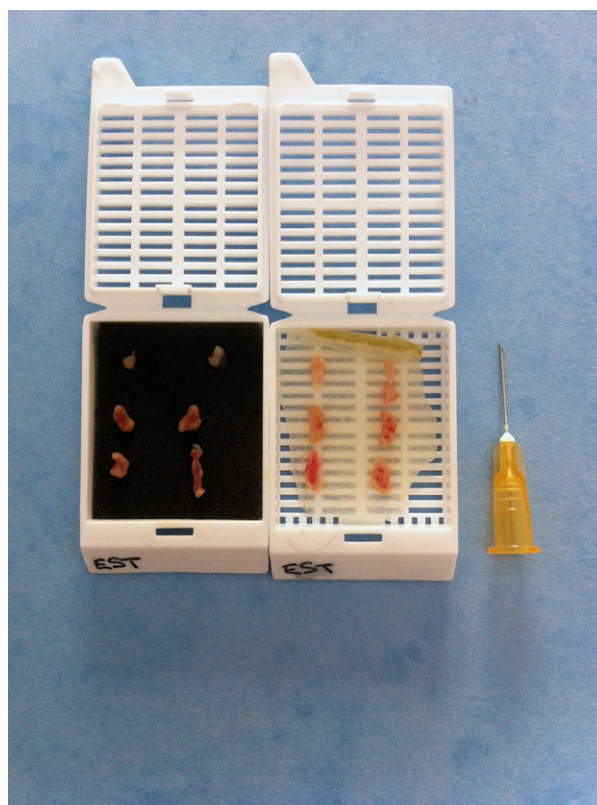


Fig 1. Gastric endoscopic biopsies following mounting and orientation on the synthetic foam sponge (left cassette) and on the thin cucumber slice (right cassette). Each line of 2 biopsy specimens corresponds to 1 site of the stomach (antrum, body, and fundus from the top to the bottom, respectively).

slices then were dehydrated in a 90% medical spirit bath, changed daily for 3 consecutive days, and then conserved in medical spirit at room temperature until use. The slices were removed from the spirit jar, wiped dry, and adapted in size to the cassette just before the biopsy process. After endoscopy, the clinician reported the examination according to WSAVA guidelines,⁸ and recorded positioning and adherence of the samples to the mount (sponge and cucumber slice) as adequate, intermediate, or inadequate.

Laboratory process

On arrival at the laboratory, concordance of sample numbers was assessed and recorded for each technique by a technician. Floating free specimens and specimens mounted on sponge were gently handled with forceps and embedded in paraffin. The cucumber slice was handled directly with the forceps and was embedded, together with the specimens attached to it, in paraffin. After routine preparation of 4- μ m sections for microscopy, all slides were analyzed by the same board-certified pathologist (ERG), following a standardized questionnaire (Figs 2 and 3). Sample orientation was assessed as to whether the specimen reached the mucosa only, muscularis-mucosa, or submucosa. Sample width was measured directly on the histopathologic slide at the maximum dimension in any direction using a ruler. The presence of artifacts was reported as "absent" if no or negligible artifacts were observed, "moderate" if artifacts were present but the slide was still interpretable, or "severe" if the presence of artifacts precluded further interpretation of the slide. The last criterion was a self assessment of the

File number: _____		Histopathological examination		
Laboratory number: _____				
		Date: ____/____/____		
<i>S = stomach ; D = duodenum ; X = stomach AND duodenum</i>		Cucumber	Sponge	Floating free
# TECHNIQUE				
Concordance of the number of biopsies between endoscopy and laboratory (yes/no):				
# READING				
Orientation: (= gastrointestinal wall observed up to)	Mucosa			
	Muscularis-mucosa			
	Submucosa			
Biopsy width (mm):	Stomach			
	Duodenum			
Presence of artifacts:	Absent (negligible)			
	Moderate (but slide interpretable)			
	Severe (slide not interpretable)			
Type of artifacts:				
# INTERPRETATION				
Confidence in histopathological interpretation: (given biopsy quality)	Good			
	Intermediate			
	Fair			
# OTHER COMMENTS				

Fig 2. Questionnaire filled by the pathologist for all the cases.

pathologist’s confidence in his histopathologic diagnosis, given the sample size, quality, presence of sufficient mucosal anatomical structures, and the lesions observed.

Statistical analysis

Specimen positioning on sponge and cucumber and its adherence to the mount were compared using McNemar’s test. The 3 techniques (cucumber, sponge, and floating free specimens) then were compared for concordance of sample numbers between endoscopy and laboratory, accurate orientation of the specimens, presence of artifacts and pathologist’s confidence in the diagnosis using Cochran’s Q test. The widths of the biopsy specimens were compared using Friedman test. The techniques were compared, firstly including all samples, and then divided into gastric and duodenal subpopulations. Finally, the proportions of normal and abnormal samples showing artifacts were compared using Kendall’s W test. For all tests, the difference was considered statistically significant for a P-value of <.05. Statistical analysis was performed using a commercial statistical package.^c

Results

Twenty-three patients, consisting of 21 dogs and 2 cats, met the inclusion criteria. Median age was 5.9 years (range, 10 months to 13 years), and median weight for dogs was 13 kg (mean, 16.3 kg; range,

3.5–49.5 kg); cat weights were 4 and 4.2 kg, respectively. Gastric biopsy specimens were collected in all 23 animals. Duodenal biopsies were collected in 19 cases, because the pylorus could not be intubated in 4 cases. In total, 88 different sites were sampled to obtain 528 specimens. Although biopsy specimens were missing from 2 cassettes with the cucumber technique and from 1 cassette with the sponge technique on arrival to the laboratory, this finding was not statistically significant considering the overall concordance between sample numbers. Final diagnoses for all cases are summarized in Table 1.

With respect to the positioning and adherence of the samples to the mounts, as assessed by the clinician during endoscopy, the cucumber slice was considered significantly better than the sponge (adherence considered good for 19/23 versus 7/23 cases, respectively; P = .012). The biopsy specimens adhered better to the cucumber and could be stretched out and oriented, with the outer surface of the mucosa against the cucumber. However, this did not affect the orientation of the specimens on histopathology for which there was no statistical difference among the 3 techniques regardless of the region of the upper gastrointestinal tract (Table 2).

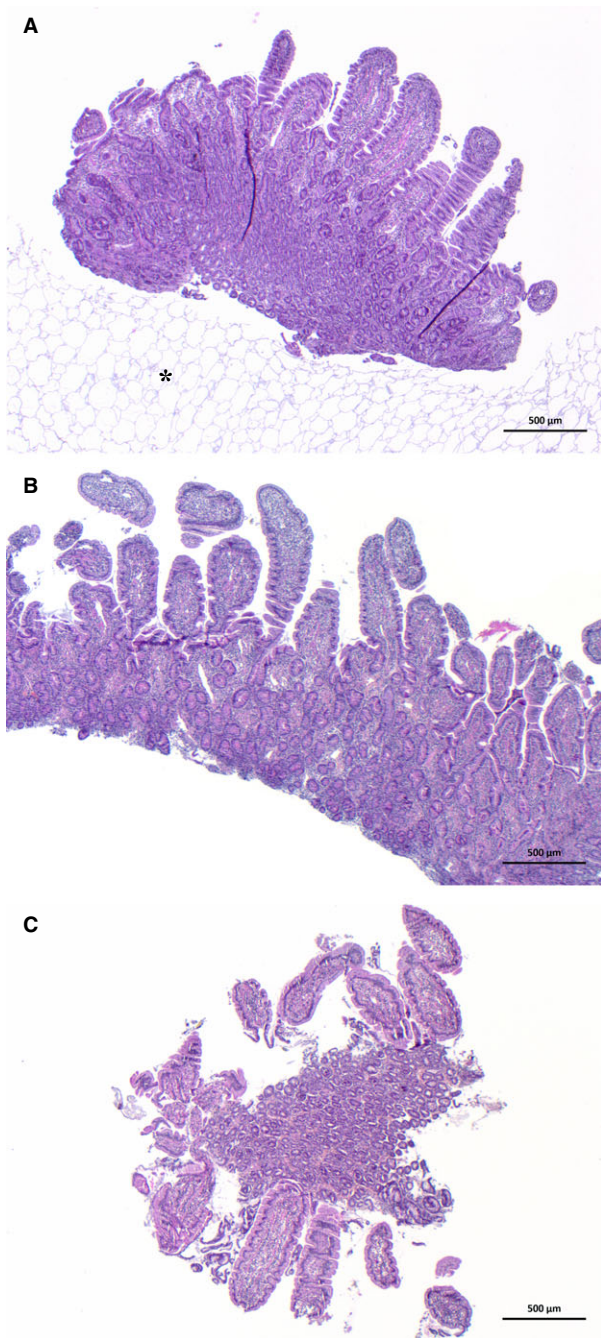


Fig 3. Histopathological sections of the duodenum from the same dog using (A) cucumber slice, (B) synthetic foam sponge, and (C) floating free techniques. The cucumber has been included in the paraffin block and is recognized on the slide (*). Note the size variation between the 3 methods. The small size of floating free biopsies resulted in inappropriate orientation and crush artifacts. Bar = 500 µm. Hematoxylin, eosin and saffron stain.

The widths of the samples for the stomach and duodenum are presented in Table 3 for all 3 techniques. Overall, the samples were considered wider on cucumber and sponge compared to those floating free in formalin ($P < .001$ and $P = .001$, respectively). This also was the case when considering the gastric subpopulation

Table 1. Histologic diagnosis for the 23 cases.

	Stomach	Duodenum
Within normal limits	12	7
Inflammatory (lymphoplasmacytic, eosinophilic, granulomatous)	3	9
Lymphoid follicular gastritis	6	N/A
Fibrosis	1	0
Lymphoma	1	1
Lymphangiectasia/lacteal dilatation	N/A	2
Total	23	19

N/A, not applicable.

only ($P < .001$ and $P = .004$, respectively). Considering the duodenal subpopulation, the difference was only statistically significant between cucumber and floating free techniques ($P = .007$); there were no significant differences in width between the cucumber and sponge techniques.

The presence of artifacts (“moderate” and “severe” as opposed to “absent”) was assessed for the 3 techniques (Table 2). Considering all samples, there were significantly fewer artifacts with the sponge compared to cucumber (artifacts absent in 36/42 versus 30/42 cases, respectively; $P = .05$), and compared to samples floating free in formalin (artifacts absent in 36/42 versus 29/42 cases, respectively; $P = .02$). Focusing on gastric samples, this difference was statistically significant between sponge and samples floating free (artifacts absent in 23/23 versus 18/23 cases, respectively; $P = .012$) and between cucumber and samples floating free (artifacts absent in 22/23 versus 18/23 cases, respectively; $P = .046$). No statistical difference was observed among the 3 techniques when considering the duodenal subpopulation only. Observed artifacts included mainly crushed and fragmented biopsy specimens (present for all 3 techniques), and small and retracted samples (mainly for floating free specimens).

The pathologist’s confidence in his interpretation and diagnosis after examination of the histopathologic slides was compared among the 3 techniques (Table 2). Considering all samples, confidence was significantly greater with the sponge technique compared to specimens floating free in formalin (confidence considered good in 37/42 versus 27/42 cases, respectively; $P = .002$). Focusing on gastric samples, this difference was statistically significant between sponge mounted and floating free specimens (confidence considered good in 23/23 versus 17/23 cases, respectively; $P = .005$) and between cucumber mounted and floating free specimens (confidence considered good in 22/23 versus 17/23 cases, respectively; $P = .021$). No statistical difference was observed among the 3 techniques when considering the duodenal subpopulation only.

The samples were reviewed at the end of the study process to assess whether the presence of histopathological lesions had an influence on the presence of artifacts. All 525 specimens were determined to be either “within normal limits” or “abnormal” (i.e., contained histopathologic lesions); whether they had artifacts or

Table 2. Comparative results of the 3 techniques of histopathologic analysis and interpretation.

	Stomach (23 Cases)			Duodenum (19 Cases)			Total (42 Cases)		
	Cucumber Slice	Synthetic Sponge	Floating Free	Cucumber Slice	Synthetic Sponge	Floating Free	Cucumber Slice	Synthetic Sponge	Floating Free
Orientation									
Mucosa	16	17	18	18	19	17	34	36	35
Muscularis-mucosa	7	6	5	1	0	2	8	6	7
Submucosa	0	0	0	0	0	0	0	0	0
Presence of artifacts									
Absent	22 ^a	23 ^b	18 ^{a,b}	8	13	11	30 ^c	36 ^{c,d}	29 ^d
Moderate	1	0	4	8	4	4	9	4	8
Severe	0	0	1	3	2	4	3	2	5
Confidence in interpretation									
Good	22 ^f	23 ^g	17 ^{f,g}	9	14	10	31	37 ^e	27 ^e
Intermediate	1	0	5	7	3	5	8	3	10
Fair	0	0	1	3	2	4	3	2	5

a,b,c,d,e,f,g Difference considered statistically significant between the techniques.

Table 3. Width of the endoscopic biopsies (in mm) for the different techniques (median [minimum; maximum])

	Cucumber Slice	Synthetic Foam Sponge	Floating Free Biopsies
Stomach	4.00 [2; 6] ^a	3.00 [2; 5] ^b	2.50 [1.5; 4] ^{a,b}
Duodenum	3.00 [2; 5] ^c	3.00 [1.5; 5]	2.00 [1.5; 5] ^c
All samples	3.75 [2; 6] ^d	3.00 [1.5; 5] ^e	2.00 [1.5; 5] ^{d,e}

a,b,c,d,e Difference considered statistically significant between the techniques.

not also was recorded (Table 4). There were significantly more artifacts in the population of samples considered normal compared to the population of samples with pathological lesions (15.3% versus 10.7% of the samples had artifacts, respectively; $P = .014$). Considering the 3 techniques individually, the difference was only statistically significant for the samples floating free in formalin (22.8% versus 10.0% of samples had artifacts, respectively; $P = .003$).

Discussion

Endoscopy and biopsies of the gastrointestinal tract are performed frequently in dogs and cats, and numerous studies have been published on the technique itself and the interpretation of histopathologic specimens by the pathologist. However, the present study is, to our knowledge, the first to compare different techniques to handle the specimens from their sampling to their processing in the laboratory.

As already observed in preliminary work conducted at our institution, the present study has confirmed the superiority of cucumber slices over moisturized synthetic foam sponges to mount and orient endoscopic biopsy specimens. The sample adhered easily to the cucumber surface, allowing proper display and orientation of the specimen with the help of a 25G hypodermic needle.¹⁴ Furthermore, the samples on cucumber slices were significantly wider than those

floating free in fixative. This finding was expected and is consistent with that of another study, because sample adherence to the mount maintains elongation during fixation with formalin, whereas free-floating samples tend to retract and curl up, leading to shorter specimens with distorted architecture on histopathologic slides (Fig 4).^{3,13} Similarly, samples on synthetic sponge were significantly wider compared to those floating free in fixative. However, despite the attention paid to orient the specimens properly on the cucumber slice and foam sponge during endoscopy to avoid tangential sections, we failed to demonstrate that these techniques resulted in deeper samples on histopathology. The reason for this finding is unclear. Different technicians with variable training in sample processing were involved in our laboratory, which precluded complete standardization. This situation could have contributed to limiting the benefit of these techniques in our study. In addition, a discrepancy had been noticed among the number of samples encased in cassettes during endoscopy and the number retrieved in the laboratory, but this only affected a limited number of cases and was not statistically significant. We used different cassette models as a consequence of supplier changes during the course of the study, and we assumed that some cassette fenestrations were wide enough to allow the samples to move, and potentially pass through them.

Endoscopic biopsy specimens are of small size and are prone to damage, in particular crush artifacts, during handling and carriage from endoscopic collection to laboratory processing. In particular, the samples must be manipulated with forceps to be oriented for embedding in paraffin. Conversely, cucumber slices can be handled directly and embedded in paraffin during laboratory processing, keeping the same orientation without damaging the sample itself. Nonetheless, in our study, the presence of artifacts was significantly lower on samples on synthetic sponge compared to samples on cucumber slice or floating free in formalin. All biopsy specimens were handled

Table 4. Proportion of “normal” and “abnormal” samples with artifacts (moderate or severe).

	Number of Biopsies “Within Normal Limits”				Proportion of Biopsies “Within Normal Limits” with Artifacts				Number of Biopsies with Histopathologic Lesions				Proportion of Biopsies with Histopathologic Lesions with Artifacts																																		
	Cucumber		Synthetic Sponge		Floating Free		All 3 Techniques		Cucumber, %		Synthetic Sponge, %		Floating Free, %		All 3 Techniques, %																																
	106	19	23	124	109	27	136	385	7.5	52.6	14.4	3.0	13.8	8.2	31	18	49	37	14	51	29	11	40	97	43	140	3.2	38.9	16.3	0.0	21.4	5.9	10.3	9.1	10.0	4.1	25.6	10.7									
Stomach																																															
Duodenum																																															
Total																																															

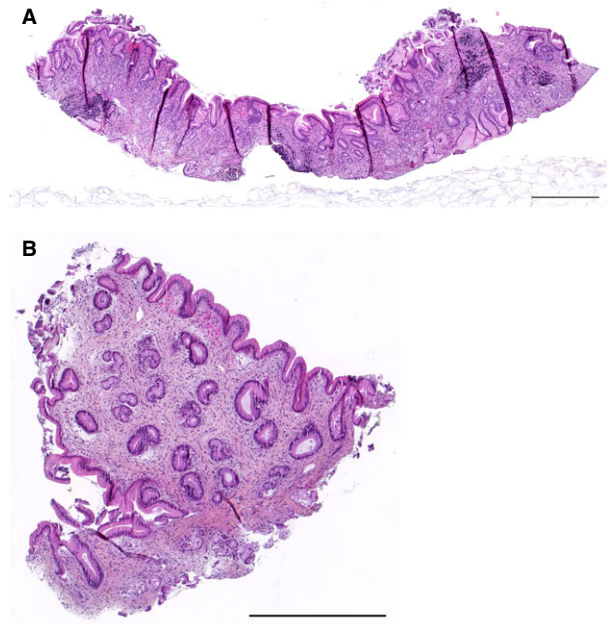


Fig 4. Histopathologic sections of the stomach from the same dog using (A) cucumber slice and (B) floating free techniques. The specimen on cucumber appears wide and well oriented, whereas the floating free specimen is retracted. Bar = 1000 µm. Hematoxylin, eosin and saffron stain.

gently with a hypodermic needle to retrieve them from the endoscopic forceps and improve their orientation on both cucumber slice and synthetic sponge. Although using a needle could have caused some artifacts compared with gentle agitation of the forceps in formalin, it was required to mount the samples correctly on both cucumber and sponge. Because all samples were processed in the same manner, this is unlikely to have caused more artifacts for 1 technique as compared to the others. In contrast, cucumber slices were conserved in medical spirit before use. Although they were dried with absorbent tissue before sample collection, residual presence of spirit on the slice might have caused excessive specimen dehydration and secondary artifact. Ethanol is known to cause morphological changes in intestinal biopsy specimens *in vivo*.²⁰ In addition, samples mounted on sponge and cucumber were encased in small plastic cassettes before immersion in formalin, which prevented excessive movements of the specimens during carriage to the laboratory compared to free-floating specimens. Finally, retrieval of the samples from formalin with forceps for embedding in paraffin can be challenging because of their small size and pale color, whereas the samples already placed on the dark synthetic sponge were considered easier to retrieve and embed in paraffin.

Overall confidence in the diagnosis was considered statistically superior with the synthetic sponge technique over specimens floating free in formalin. This criterion was chosen to assess whether the observed differences between the techniques had an effect on the ability of

the pathologist to give a reliable diagnosis. Although subjective and pathologist dependent, we consider these results relevant in our study, because the same pathologist reviewed all slides and therefore applied the same scoring system to all specimens, although the study could not be blinded. The degree of confidence generally correlated with the severity of artifacts, which were less important with the synthetic sponge. This impact of sample quality on histopathological diagnosis already has been reported in previous studies.^{2,3} Considering all of our data, it appears that the pathologist's confidence in the diagnosis was more influenced by the presence of artifacts than by the width or orientation of the samples. This observation is interesting and could guide the choice of endoscopic forceps.⁷

We also compared our results with regard to the biopsy site, between stomach and duodenum. We initially hypothesized that our findings would vary because of differences between the gastric and duodenal mucosa and the presence of villi in the duodenum, making the orientation more critical in the duodenal specimens. However, no statistical difference was identified between the techniques for the duodenal subpopulation regarding the presence of artifacts and the degree of confidence in the histopathologic diagnosis.

A common assumption is that samples with histopathologic lesions will be more friable and fragile, and that their collection and handling will more likely be associated with artifacts. Interestingly, we found that samples with no histological lesion were more likely to have artifacts. The difference was significant ($P = .003$) when considering only specimens floating free in formalin. The reason for this observation is unclear. However, it strengthens the case for using mounts to process endoscopic biopsy specimens.

Our study has several limitations. The cassette model changed during the study period, and this is the most likely cause of some specimen loss for the cucumber (2/176) and sponge (1/176) techniques, although this change affected very few samples. More importantly, different technicians were involved in the laboratory process and this has been associated with a lack of standardization of specimen management. Specific training of technicians with cucumber slices and the development of process protocols may improve the quality of histopathologic slides with better orientation and better diagnostic confidence. Furthermore, the pathologist could not review the slides blindly, because all 3 techniques had different characteristics making them easily identifiable. Because of animal welfare considerations (eg, duration of anesthesia, number of biopsies taken per site), we limited our comparison to 3 methods, which were considered different and representative of the techniques most commonly recommended in the literature. Other studies including other media, such as filter or cellulose acetate paper could be performed in the future.^{12,13} Furthermore, we have limited our study to the upper gastrointestinal tract, and further studies focusing on the lower gastrointestinal tract or other organs (eg, urinary, genital or respiratory tracts) would be warranted.

Conclusions

In conclusion, our study has demonstrated the superiority of mounted biopsy specimens of the upper gastrointestinal tract over specimens floating free in formalin with regard to their handling from collection to processing in the laboratory. Specimens mounted on moisturized synthetic foam sponge had fewer artifacts and were associated with the highest confidence of the pathologist in the analysis and interpretation of the specimens. However, involvement of different technicians and inconsistency in cassette usage preclude definitive conclusions regarding whether or not synthetic foam sponge should be preferred over cucumber slices. Moreover, technical processing of the specimens can influence the results and standardizing these protocols is important. Additional studies are needed to investigate other mounting media and to document the influence of the mount on endoscopic biopsy specimens from other locations.

Footnotes

^a GIF-160 gastroscope, Olympus, Tokyo, Japan

^b FB-210K forceps, Olympus, Tokyo, Japan

^c SPSS software (Statistical Package for Social Sciences) version 23.0.0.2, IBM Statistics, Chicago, IL, USA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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