

# Routine Chromoendoscopy for Gastrointestinal Diseases: Indications Revised

M. Kida  
K. Kobayashi  
K. Saigenji

## Introduction

Can we be sure that we have identified every lesion after an endoscopic examination? We may believe we have seen all of the sites that need to be examined – but what percentage of the cancers diagnosed are early ones? In our department, some 50–60% of lesions identified are early gastric cancers, but this still means that 40–50% of lesions found represent advanced gastric cancer. Of course, the principal reason for late diagnosis is late presentation; however, a proportion of cases are likely to be due to lesions being overlooked during endoscopy.

Endoscopic treatment methods such as endoscopic mucosal resection (EMR), photodynamic therapy (PDT), and argon plasma coagulation (APC) are now meeting with acceptance throughout the world as radical methods of treating early esophageal and gastrointestinal cancers. These methods have the advantages of reduced invasiveness, improved cost-effectiveness, and shorter hospital stays. However, if it is not possible to identify early cancers conclusively as an indication for endoscopic therapy, then such treatments cannot be offered. Early cancers – generally involving flat, but not always small, lesions – need to be detected more frequently. Chromoendoscopy first came into clinical use some 40 years ago as a method of identifying lesions and studying them in detail, and several types of chromoendoscopy stain have since been developed (Table 1). These stains can be extremely helpful for identifying early cancers, and they are discussed in detail below.

## Indigo Carmine

Indigo carmine staining is a contrast method that highlights irregularities in the mucosal architecture as a result of pooling of the blue dye solution in mucosal grooves and depressed areas. This is the dye most frequently used for chromoendoscopy in Japan [1]. Indigo carmine was originally developed as an agent for evaluating renal function by intravenous injection. In chromoendoscopy, it is applied either directly or indirectly.

In the direct method, a 0.2% (0.1–0.4%) solution of indigo carmine is sprayed, after standard endoscopic observation, onto an area that shows changes such as altered coloring, mucosal irregularity, ulceration, or an obscure vascular network (Figures 1, 2). In the indirect method in the stomach, 10 ml of 3% indigo carmine solution is administered orally after the patient has taken a solution (consisting of dimethylpolysiloxane, proteinase enzyme, and sodium bicarbonate) to clean the gastric mucus [1–3]. In the indirect method in the colon, a 100-mg capsule of indigo carmine dye powder is taken orally [4], or a cup of 20–40 mg of indigo carmine is taken, immediately diluted with water, following administration of 2 l of a polyethylene glycol electrolyte lavage solution several hours before colonoscopy [5]. Although reports have described a high rate of detection of flat, depressed, or elevated lesions with the indirect method [1,6], the direct method with a spraying catheter is commonly used in Japan. The indirect method has the disadvantage that standard endoscopic observation is not possible without washing.

Endoscopic observation with indigo carmine is used to visualize lesions in detail, and the indications for using this method to examine malignant lesions are:

### Institution

Dept. of Gastroenterology, Kitasato University East Hospital, Sagami-hara, Japan

### Corresponding Author

M. Kida, M.D. · Dept. of Gastroenterology · Kitasato University East Hospital · 2-1-1 Asamizodai, Sagami-hara · Kanagawa 228-8520 · Japan · Fax: +81-42-749-8690 · E-mail: m-kida@kitasato-u.ac.jp

### Bibliography

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Table 1 Routine chromoendoscopy stains in gastrointestinal endoscopy

Stain type	Main organs	Mechanism	What stains	Positive stain	Clinical aims
<b>Contrast stains</b>					
Indigo carmine	Stomach, colon, small intestine	Pools in mucosal grooves and depressed areas	Cells do not stain; only pooling effects	Blue	Screening of early cancer; differentiation between malignant and benign lesions; pit-pattern analysis; diagnosis of spread and depth; observation of inflammatory diseases; evaluation of ulcer healing
<b>Vital stains</b>					
Lugol's solution	Esophagus	Binds iodine in nonkeratinized cells	Normal squamous cells with glycogen	Dark brown	Screening of early esophageal cancer; reflux esophagitis; columnar epithelium in the esophagus; follow-up after mucosal ablation in Barrett's esophagus
Methylene blue	Esophagus, small intestine, colon	Active absorption into cells	Small- and large-intestinal cells, intestinal metaplasia	Blue	Specialized epithelium in Barrett's esophagus; intestinal metaplasia in the stomach; ectopic gastric mucosa in the duodenum
Toluidine blue	Esophagus	Diffusion into cells	Binds to cellular nuclei	Blue	Squamous-cell carcinoma; reflux esophagitis; gastric or intestinal metaplasia in Barrett's esophagus
<b>Reactive stains</b>					
Congo red	Stomach	pH < 3 results in color changes	Acid-secreting gastric cells	Turns red to blue-black	Mapping of acid-secreting gastric mucosa; diagnosis of ectopic gastric mucosa
Phenol red	Stomach	Alkaline pH results in color changes	<i>H. pylori</i> -infected gastric mucosa	Turns yellow to red	Diagnosis of <i>H. pylori</i> infection

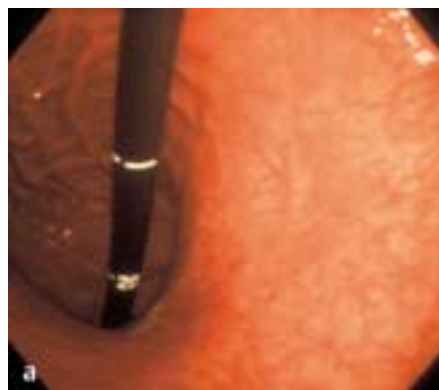


Figure 1 a,b A flat, elevated early gastric cancer (0-IIa), stained with indigo carmine.



Figure 2 a,b A depressed-type early gastric cancer (0-IIc), stained with indigo carmine.



- For screening of early cancer (mainly in the stomach and colon)
- To differentiate between benign and malignant lesions
- For magnifying endoscopy with pit-pattern analysis (mainly in the colon)
- To diagnose the extent of spread of superficial cancer
- To diagnose the depth of cancer invasion

If chromoendoscopy techniques such as indigo carmine staining had not been introduced, it is possible that Japanese endoscopists might not have the reputation for expertise in diagnosing and managing early gastric cancers which they currently enjoy. In comparison with standard endoscopy, indigo carmine chromoendoscopy allows improved early detection of malignant

signs such as irregular margins, depressed areas (type 0-IIc) next to ulcerations, and narrowing, interruption, or swelling of converging folds [7]. Since the first report (by Kudo's group) of a case of depressed-type early colon cancer, indigo carmine chromoendoscopy has mainly been used with magnifying endoscopy for pit-pattern analysis, because of its convenience and efficacy [6,8,9].

The indications for indigo carmine staining in benign lesions are:

- Observation and evaluation of inflammations and ulcerative lesions such as those seen in Crohn's disease, ulcerative colitis, healing peptic ulcers, celiac disease, tropical sprue, and Barrett's esophagus
- Detection of small lesions
- Differentiation between gastric antral and fundic glands

Indigo carmine staining has been used to diagnose villous atrophy in patients with celiac disease or tropical sprue [10,11], and in combination with magnifying endoscopy to diagnose a villous appearance in Barrett's esophagus [12]. In their paper in the present issue of *Endoscopy*, Kiesslich et al. conclude that staining of the duodenum with indigo carmine is useful for detecting mucosal abnormalities and allowing targeted biopsies, although magnifying endoscopy does not appear to increase the diagnostic yield further for detecting duodenal abnormalities [11].

### Lugol

Lugol's solution, named after the French physician J. G. A. Lugol (1786–1851) [13], has an affinity for glycogen in nonkeratinized squamous epithelium. Lugol's iodine solution mainly causes dark brown staining of nonkeratinized squamous epithelium, which has glycogen-rich granules in the prickle-cell layer (stratum spinosum). This iodine stain was first used to detect cervical cancer by Schiller, and was used to detect esophageal cancer by Voegeli in 1966 [14]. Approximately 10–30 ml of Lugol solution, at concentrations ranging from 0.5% to 4.0%, is sprayed after the esophagus has been washed with 20–60 ml of water. We use a 1.5% solution for routine screening and a 3.0% solution for pre-operative evaluation. After Lugol spraying, the esophagus is washed with 20–40 ml of water again, and a search for lesions is then carried out. Defects in the normal esophageal epithelium – such as those caused by esophageal cancer, dysplasia, or esophagitis – do not stain (Figure 3). With standard endoscopy, it is generally difficult to identify superficial esophageal cancers (in contrast to gastric cancers) suitable for EMR, which is used to treat lesions limited to the epithelium (m1) or to the lamina propria (m2). Lugol chromoendoscopy should therefore be used routinely in at-risk patients – those with head and neck cancer [15,16], heavy smokers, heavy drinkers, men over 50 years old [17,18], patients with a mutant aldehyde dehydrogenase 2 (*ALDH2*) allele [19], and those with multiple glycogenic acanthosis [20]. There have been several reports of a high detection rate for superficial esophageal cancer or dysplasia using Lugol chromoendoscopy [17,18,21]. Several unstained areas are sometimes seen after Lugol spraying; in benign lesions, the unstained area has a rounded appearance, while malignant unstained areas have irregular margins and are generally over 10 mm in diameter.

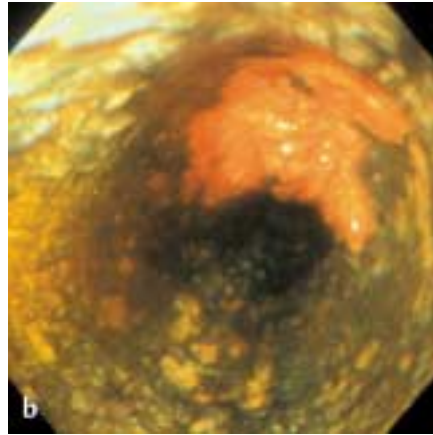


Figure 3a,b A slightly depressed esophageal cancer (0-IIc), stained using Lugol chromoendoscopy.

Lugol is an irritant that damages the normal epithelium, and patients therefore often complain of abdominal pain, heartburn, and nausea. In addition, allergic reactions to iodine, including shock, sometimes occur. Administration of 20 ml of a sodium thiosulfate solution, which substantially reduces these adverse events, is therefore recommended after Lugol chromoendoscopy [22]. A subsequent Lugol chromoendoscopy examination can only be carried out after an interval of at least 3–4 weeks, due to epithelial damage. In the double-staining method using Lugol and toluidine blue, toluidine blue has to be sprayed first, followed by Lugol – otherwise the toluidine blue stains not only the lesion but also the normal epithelium, which is damaged by the Lugol. The original Lugol solution contained glycerin, but for staining of the esophageal epithelium, an iodine solution without glycerin appears to be better due to its low viscosity and better staining characteristics [17].

### Methylene Blue

Methylene blue staining was first described by Ida et al. [23] as a technique for improving the diagnosis of early gastric cancer. Methylene blue is a vital stain that is taken up by absorbent tissue such as the small-intestinal and colonic epithelium, in contrast to the nonabsorptive squamous and gastric mucosa. A total of 10–20 ml of methylene blue (0.1–0.5%, generally 0.2%) is sprayed with a spraying catheter directly over the mucosa, and after 1–2 min the mucosa is washed with water to allow examination of the staining effects.

The indications for methylene blue chromoendoscopy are: to detect Barrett's epithelium (positive staining); to diagnose ectopic gastric mucosa in the duodenum (negative staining) (Figure 4); and to diagnose adenoma or cancer in the colon (little or no staining) [1].

In Western countries, Barrett's esophagus is of clinical importance due to its high prevalence and potential for carcinogenesis. Barrett's esophagus includes three different types of metaplastic replacement of squamous epithelium in the esophagus – the fundic type, the cardia type, and the intestinal type known as specialized columnar epithelium (SCE). It is generally accepted that only the last type, SCE, carries a risk of carcinogenesis. Barrett's esophagus is defined endoscopically as columnar-lined mucosa extending for more than 2–3 cm from the stomach and lying on the longitudinal vessels in the lower esophagus. Columnar-lined mucosa extending for less than 2–3 cm is known as short-segment Barrett's esophagus [24]. The distal end of the longitudinal vessels in the lower esophagus, which are seen during endoscopy under the transparent columnar epithelium, is considered to represent the original esophagogastric junction.

Canto et al. were the first to report that methylene blue selectively stains only the intestinal type of Barrett's esophagus, specialized columnar epithelium [25]. In Barrett's esophagus, methylene blue stains either focally or diffusely (over 75% of Barrett's mucosa stains blue). The majority of patients with long-segment Barrett's esophagus have diffuse staining, as in these cases SCE makes up most of the columnar mucosa. Dysplasia is significantly associated with focal areas of reduced staining intensity and/

or increased heterogeneity of the stain. High-grade dysplasia and adenocarcinoma in Barrett's esophagus can also be detected as heterogeneously stained or light blue/unstained epithelium. However, the efficacy of methylene blue staining for diagnosing Barrett's esophagus and detecting adenocarcinoma has remained a matter of controversy [25–27]. The variability in the reported results may be due to the quality of the washing preparation and individual differences in staining characteristics in patients with Barrett's esophagus.

Ectopic gastric mucosa in the duodenum appears as an unstained area in blue-stained duodenal mucosa (Figure 4a,b); methylene blue has therefore been used as a method of screening for small colonic lesions, which remain unstained in contrast to the blue-stained colonic mucosa [1].

### Toluidine Blue

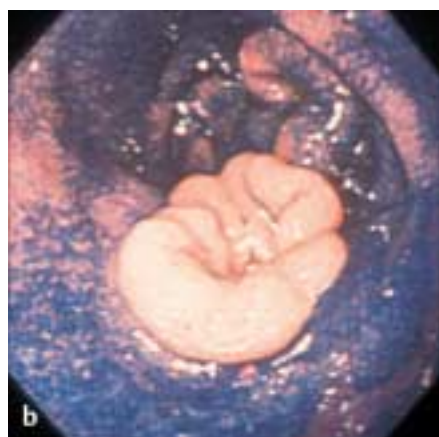
Toluidine blue is an acidophilic metachromatic stain that binds to cellular nuclei and was originally used in the management of diseases of the oral cavity [28]. Cells with increased DNA synthesis and a high nuclear–cytoplasmic ratio tend to absorb this stain more intensely [3]. A 2% solution (or sometimes a 1% solution) of toluidine blue is used after the esophagus has been washed with water or acetic acid (as food residues may also take up the stain). Toluidine blue chromoendoscopy is mainly used to detect superficial esophageal cancers and assess their depth of invasion. Epithelial cancers limited to the basal layer and Japanese “dysplasia” lesions mostly do not stain, and contrast with areas unstained by Lugol; other epithelial cancers appear as a light blue violet; m3 lesions (with invasion into the muscularis mucosae) appear as a blue violet; and cancers with submucosal or deeper invasion appear as dark blue. However, toluidine blue stains not only esophageal cancers, but also esophageal ulcers and areas of esophagitis. For preoperative evaluation of superficial esophageal cancers, a double-staining method using toluidine blue and Lugol is sometimes used, with toluidine blue being sprayed first, followed by Lugol.

Toluidine blue can also stain columnar mucosa in Barrett's esophagus, but it is not capable of differentiating between intestinal and gastric metaplasia. When toluidine blue was first introduced as a chromoendoscopy stain, it was also used to distinguish between benign and malignant gastric ulcers. This indication has in the meantime been replaced by indigo carmine, as toluidine blue provides poorer contrast to the gastric mucosa and is less convenient to use than indigo carmine, because of a staining method.

Adverse events such as abdominal pain and nausea have been reported with toluidine blue, and amounts of 10–20 ml or less are therefore recommended.



Figure 4a,b Ectopic gastric mucosa, stained using methylene blue chromoendoscopy.



## Cresyl Violet

Cresyl violet, which has been used to stain cervical lesions [29], is an in-vitro stain used for histological identification of *Helicobacter pylori*. It is reported to highlight the mucosal pattern of gastric neoplasia [30]. Since the introduction of Kudo's pit-pattern criteria for assessing colonic lesions, cresyl violet has also been used, along with crystal violet, to diagnose colonic neoplasms. Generally, a small amount (1–2 ml) of 0.1% cresyl violet is used for staining, in order to avoid excessive darkening of stained surfaces [9].

## Crystal Violet

Crystal violet (gentian violet) is a topical antimicrobial agent that binds to microbial DNA and also to the nuclei of eukaryotic cells. It was initially used to measure pH on the surface of the gastric mucosa [31]. Crystal violet stains intestinal metaplasia as well as cancerous lesions. It has been reported that the use of crystal violet after methylene blue staining reveals the detailed mucosal structure of early adenocarcinoma arising from Barrett's esophagus [32]. Recently, crystal violet has been used to diagnose colonic lesions, along with cresyl violet, following the introduction of Kudo's pit-pattern criteria for assessing colonic lesions (Figure 5). Generally, a small amount (1–2 ml) of 0.05% crystal violet is used for staining, in order to avoid excessive darkening of stained surfaces [9]. Crystal violet can be stored at normal room temperature for a day or two after opening, and needs to be renewed regularly.

## Congo Red

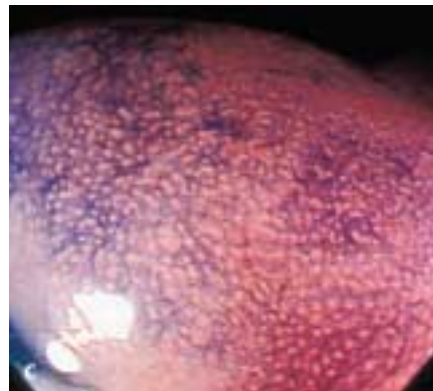
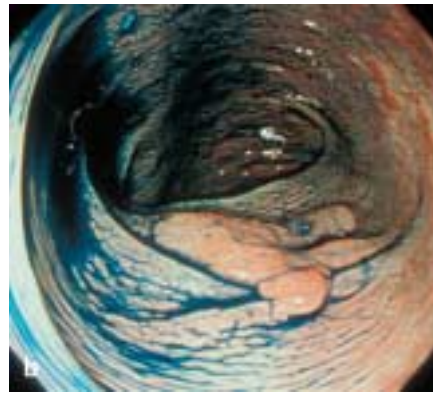
Congo red, which was introduced by Chida et al. in 1966 [33], is a pH indicator. It changes from red (above pH 5) to a dark blue-black in acidic conditions (under pH 3). The technique of Congo red staining involves initial spraying of 0.5% sodium bicarbonate; 0.3–0.5% Congo red solution is then sprayed on all areas of interest (e.g., in the stomach), and 250 µg of pentagastrin, which stimulates acid secretion, is injected intravenously [1]. This causes a positive reaction within a few minutes that distinguishes between acid-secreting areas and non-acid-secreting areas. Anticholinergic drugs should not be used in premedication. This stain has been used to map acid-producing areas for preoperative evaluation in patients who are about to undergo gastrectomy with vagotomy due to peptic ulcer [34]. However, its use has declined following the introduction of H<sub>2</sub>-blockers and proton-pump inhibitors, as well as *H. pylori* eradication therapy.

Other indications for Congo red staining include ectopic gastric mucosa (Figure 6). The stain used to be used to screen for gastric cancers and detect synchronous lesions, in combination with methylene blue, which stains areas of gastric intestinal metaplasia [35]. It has also been reported that early gastric cancer is usually found in a "bleached area" of mucosa that does not stain with either Congo red or methylene blue [13].

Congo red is generally a safe stain, and no adverse events have been reported in common use.



Figure 5a–c A flat, elevated adenoma in the colon, stained using indigo carmine chromoendoscopy; magnification view with crystal violet.



## Phenol Red

Phenol red, which detects alkaline pH by changes in color from yellow to red, is also a pH indicator [2,3]. In the era of *H. pylori* eradication, phenol red is a clinically promising method of detecting *H. pylori* infection in the stomach. The urease produced by the bacterium catalyses hydrolysis of urea to NH<sub>3</sub> and CO<sub>2</sub>, resulting in increased pH levels. It has been reported that endoscopic phenol red testing improved the diagnosis of *H. pylori* and mapped its distribution in the stomach with a sensitivity of 100% and a specificity of 84.6% [36].

The phenol red test requires treatment with a potent acid-suppressive agent in all patients, and application of a mucolytic agent (dimethylpolysiloxane) and an anticholinergic drug just before endoscopy [37]. A solution consisting of 0.1% phenol red and 5% urea is sprayed over the gastric mucosa. Positive areas that change from yellow to red indicate the presence of *H. pylori*.

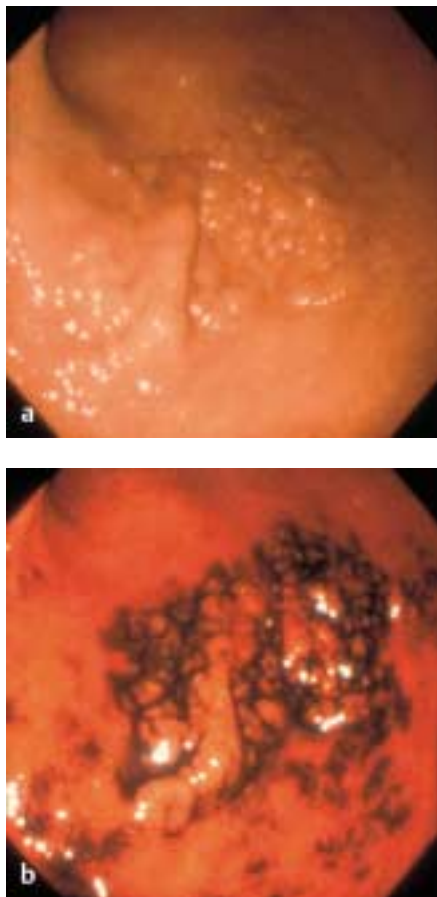


Figure 6a,b A residual fundic gland in antral intestinal metaplasia, stained using Congo red chromoendoscopy.

## Conclusions

As detailed above, chromoendoscopy is a helpful technique for diagnosing early esophageal and gastrointestinal cancers and evaluating other disorders. Its use can be recommended.

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## Other Stains

**India ink** consists of inert carbon particles suspended in aqueous or nonaqueous stabilizers and diluents [13]. Injection of India ink with a sclerotherapy needle is used for permanent marking of the location of lesions – for example, to mark colonic lesions preoperatively, or to mark malignant polyps or large adenomas for removal using piecemeal polypectomy. India ink generally remains in the gastrointestinal wall indefinitely. It tends to be used without sterilization [38], although autoclaving or gas sterilization can be recommended as the preferred method of tattooing.

**Epinephrine and prostaglandin.** Approximately 20 ml of 1 : 10 000 epinephrine and prostaglandin E<sub>1</sub> is sprayed over gastric cancer lesions showing poorly demarcated borders; in some cases – particularly in superficial, poorly differentiated adenocarcinomas – this highlights the lesions as reddened areas with well-demarcated borders [39]. This technique, known as pharmacoscopy, is based on differences in the vascular response to each agent.

**Indocyanine green**, which was originally used to evaluate liver function, is injected intravenously before infrared endoscopy examinations [40]. Infrared endoscopy with indocyanine green can identify submucosal vessels and pooling of blood flow in erosive lesions.

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