NEW STAINING SOLUTION FOR IDENTIFICATION OF *HELICOBACTER PYLORI* IN THE GASTRIC TISSUE[+]

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Abstract:

In this study an attempt was made to study the efficiency of the synthetic Brielliant blue food color powder color index (C-1. No 42090) for identification of *Helicobacter pylori* (*H.pylori*) in gastric tissue. In this study 3 types of staining solutions were prepared; each type includes 3 series of concentrations 2.5%, 5% and 10%. 1st type of staining solution consist of Brielliant blue food color powder dissolved in distilled water the 2nd type consist of blue color powder dissolved in 0.5% of aqueous solution of borax, and the 3rd type was consist of blue color powder dissolved in 0.5% of aqueous solution of boric acid.

The gastric tissue sections were stained with 3 concentrations of each type of the staining solutions and with Hematoxylin and Eosin stain as a control stain.

Best result for staining of *H.pylori,* was obtained by using the third type of staining solution with 10% concentration, these organisms were showed with dark blue color, this made their identification very easy in the gastric tissue, whereas this solution wasn't efficient for histological examination. After following up, the stained bacterial cells, it was observed to be stable in color for 18 months. According to these results, 10% concentration of the third type staining solution may be considered as an efficient staining solution for identification of *H.pylori* in the gastric tissue.

Keywords: Synthetic Brilliant blue food color powder, *Helicobacter pylori*.

المستخلص

تضمنت هذه الدراسة محاولة للاستفادة من مسحوق الأزرق البراق الصبغة الغذائية الصناعية (بدلالة اللون المرقم ٤٢٠٩) في تحضير محلول لتلوين جراثيم *H.pylori* ولتشخصيها في نسيج المعدة. حُضرت ثلاث أنماط من محاليل التلوين وكل نمط من هذه المحاليل يشتمل على محاليل ذات تراكيز متسلسلة ٢،٥ % ، ٥%، و ١٠%. حُضر النمط الأول من محاليل التلوين من اذابة مسحوق الصبغة الغذائية في الماء المقطر، أما النمط الثاني من المحاليل فقد حُضر من اذابة مسحوق الصبغة الغذائية في ٠,٥% من المحلول المائي لمادة البوركس، وأخيراً حُضر النمط الثالث من محاليل التلوين من اذابة مسحوق الصبغة الغذائية في ٠,٥% من المحلول المائي لمادة حامض البوريك.

استخدمت التسع محاليل المذكورة أعلاه ومحلول الصبغة الروتينية الهيماتوكسيلين والأيوسين في تلوين مقاطع نسيج المعدة. أفضل نتيجة كانت باستخدام محلول ١٠% من النمط الثالث لمحاليل التلوين ومن خلال المتابعة و على مدى ٣٠ شهراً اكتسبت الجراثيم لوناً ثابتاً وهو الأزرق الغامق مما سهل عملية التحري عن تلك الجراثيم في نسيج المعدة، وأعطى هذا المحلول كفاءة قليلة في اجراء فحوصات التغييرات النسيجية.

نستنتج من هذه الدراسة بأن المحلول المذكور أعلاه قد يعتبر عالي الكفاءة في التحري عن جراثيم *H.pylori* في نسيج المعدة.

Introduction:

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In 1983, many reports described the frequent association between antral gastritis and the presence of Campylobacter-like bacteria, as well as of their culture and position from patients with gastritis [1]. In the past decade, however a number of studies have confirmed that campylobacter-like bacteria were present in almost all patients with gastric and duodenal ulcer, and were generally associated with antral gastritis. A consensus regarding the major role of this bacterium, now named *Helicobacter pylori (H.pylori)* causing gastroduodenal ulceration was formally presented in 1994 [2], furthermore, in June 1994, the International agency for research on cancer working, group stated *H.pylori* plays a causal role in the chain of events leading to cancer", referring to adenocarcinoma and lymphoma of the stomach as well as the more benign mucosal-associated -associated lymphoid tissues (MALT) [3]. *H.pylori* infected mucosa characteristically exhibits signs of acute inflammation manifested by intraepithelial neutrophils, super imposed upon a background of chronic inflammation shown by lymphatic infiltration. The organism is usually evident in routine stain such as hematoxylin and eosin (H&E). In case of chronic gastritis, the organisms may not be easily seen, special stain like Giemsa stain and silver-containing stains such as Warthin-Starry and Genta stain can be used to enhance the visibility of the bacteria in the specimen [4, 5].

In this report, the synthetic Brielliant blue food color powder has been dissolved in the following solutions: Distilled water. 0.5% aqueous solution of borax, and 0.5% of aqueous solution of boric acid, each type of these solutions were prepared with series of concentrations (2%, 5% and 10%), in all attempt to prepare staining solution for identification of *H.pylori* in gastric tissue. This synthetic food color powder is available in all markets (local and foreign markets) and it's inexpensive

Brielliant blue food color powder has a color index (CI No. 42090), this number identify the dye and its chemical constitution, while the name of the dye may vary with different suppliers [6, 7].

The objective of this study is to study the efficiency of Briellient blue color powder in preparation of new staining solution for identification of ***H.pylor*** *in* gastric tissue.

Materials and Methods:

- Gastric biopsies of ***H.pylori*** infected patients were brought from Gastrointestinal and hepatic diseases center. A previous histological examination was done for each biopsy to confirm the presence of ***H.pylori*** or[9]anisms in its tissues. At least one biopsy specimen from the antrum and one specimen from corpus were obtained from each patient. Tissue samples were immediately fixed in 10% buffered formalm dehydrated.
- Brielliant blue food color powder (C.1 No. 42090) was obtained from local market.
- Hematoxylin & eosin stain was brought from Pasteur institute (control stain).

Preparation of brielliant blue staining solutions:

1st type staining solution:

Concentration	Weight of the blue color	Distilled
2%	2g	100 ml
5%	5g	100 ml
10%	102	100 ml

2nd type staining solution:

Concentration	Weight of the blue color	0.5% aqueous
2%	2g	100 ml
5%	5 g	100 ml
10%	log	100 ml

3rd type staining solution:

histological and series of concentrations (2%, 5% and 10%) of three type staining solutions of brielliant blue color powder examination.
* - Sections were stained with hematoxylin and eosin for regular histological examination and with staining solution of brielliant blue powder.
* - Nine slides were performed and stained with three types of staining solutions by the following method: Each three slides were stained with one type of staining solution which includes three concentrations (2%, 5% and 10%), each slide was performed to be stained with one concentration of staining solution. One slide was performed to be stained with H&E stain

Staining procedure:

- The slides stained with H&E stain [19] and the slides stained with the new staining solutions were subjected to the same staining procedure.
- Dewax sections in xylol, hydrate through graded alcohol and water.
- Stained sections in H&E solutions and in all new blue staining solutions for 2-4 min.
- Rinse in tap water.
- Dehydrated, clear and mount with DPX.
- Examined under oil immersion to detect ***H.pylori*** organisms.
- Follow up was done during the period between March 2004 and October 2006 by periodic histological examination (once a month), for the slides which showed reliable results in staining ***H.pylor***i organisms with new staining solutions..

Results:

- The synthetic Brilliant blue food color powder was very easy and completely dissolved in all types of solutions and without any precipitate.

Concentratio	Weight of the blue	0.5% aqueous solution of
2%	2g	100ml
5%	5 g	100ml
10%	10g	100ml

Table (1): Screen results of histological examination for all tissue sections stained with 3 types of staining solutions

Types of staining solutions	Concentrations of staining solution		
	2%	5%	10%
1st type	-ve	-ve	-ve
2nd type	-ve	-ve	-ve
3rd type	+ve	+ve	+ve

- All tissue sections stained with The 1st type of staining solutions (food color powder dissolved in distilled water) and, 2nd type of staining solution (food color powder dissolved in 0.5% aqueous solution of borax) showed negative results. The gastric tissue section were completely unstained while all tissue sections stained with the 3rd type of staining solution (food color powder dissolved in 0.5% aqueous solution of boric acid) showed positive results, stain uptake of the gastric cells and bacterial cells appeared in different levels depending on the concentration of staining solution which stained them, table (2). *H.pylori* organisms in the gastric tissue which were stained with staining solution of 10% concentration of the 3rd type of staining solution, were stained with dark blue color, this color provides the most reliable result in identification of *H.pylori* organisms in gastric tissue. Flotire (1), table (2); whereas 2% and 5% concentrations of the third type of staining solution stained the bacterial cells in the gastric tissue with lighter color which made identification of them not easy, Table (2); whereas the gastric cells which stained with the three solutions of 2%, 5% and 10% concentration of the 3rd type of staining

solutions were stained with different degree of blue color depend on the concentration of the staininc, solution and showed weak contrast in the color of the cytoplasm and the nucleus of the stained cell but these solutions were not useful to be used as histological stains, Figure (1) Table (2).

- Gastric cells stained with H&E appeared with pink cytoplasm and dark violet nucleus, while the *H.pylori* organisms were stained with pink color, Figure (2), table (2).

Table (2): A rough estimation of stain uptake for gastric and bacterial cells stained with Different concentrations of 3rd type staining solution and H&E stain.

Cells color		Concentrations of 3rd type staining solution Wt/vol			H&E stain
		2%	5%	10%	
Color of gastric cells	Cytoplasm	Light blue	Moderate blue	Dark blue	Pink
	Nucleus	Light to moderate blue	Moderate to dark blue	Dark blue to black	Dark violet
Color of ***H. pylori***		Light blue	Moderate blue	Dark blue	Pink

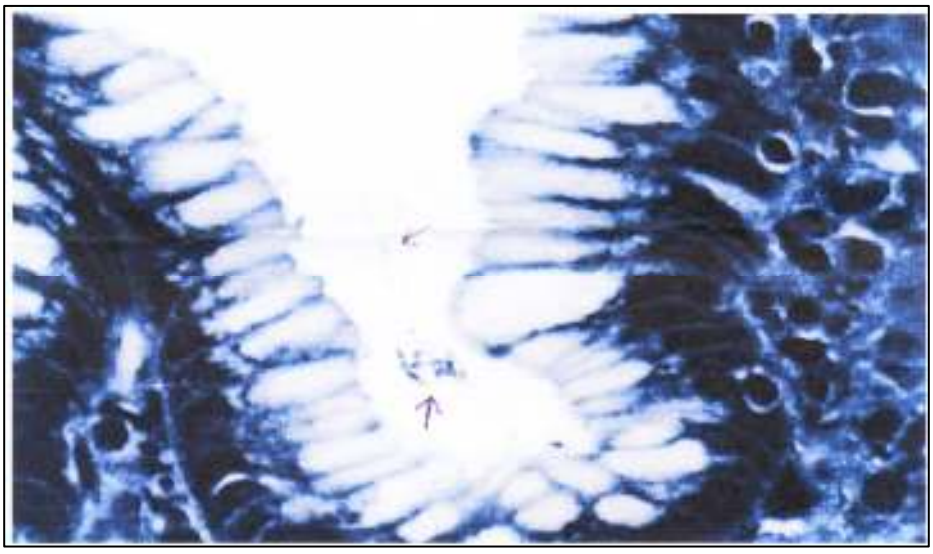
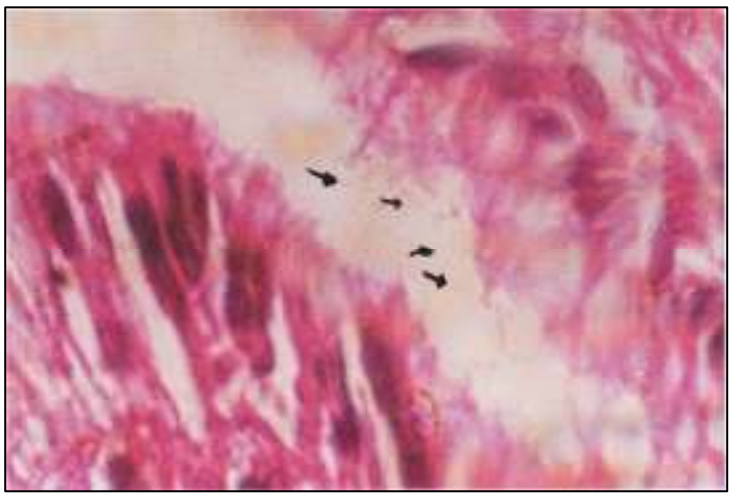


Figure (1): Photomicrograph of gastric tissue stained with a solution of 10% concentration of the 3d type of staining solution. The arrow indicates the appearance of *H.pylori* organisms stained with dark blue color (x 1000).



- After following up *H.pylori* organism which were stained with a solution of 10% concentration of the 3rd type of staining solution it appeared to be stable in a dark blue color , for 18 month .

Discussion:

In this study and through the histopathological examination. *H.pylori* organisms in the gastric tissue were present with pink color, when stained with H&E stain, pale color of *H.pylori* organisms make their recognition not easy. Many studies were reported that H&E stain alone has proven to be unsatisfactory due to the lack of sensitivity for detection of *H.pylori* organisms [4,8] other study was reported that hematoxylin a dye derived by ether extract from the wood of the Mexican tree **hematoxylin compechioman**, hematoxylin has poor staining properties and normally used in conjunction with mordant [6]. *H.pylori* organisms in the gastric tissue when stained with a solution of 10% concentration of the 3rd type of the staining solutions wee appeared with dark blue color, thus *H.pylori* organisms were very easily identified in the gastric tissue, in accordance with this results many studies were reported that the synthetic dyes are more easily controlled because of their physiochemical properties [9,10,11]. Many characteristics of this staining solution such as its color stability, and it has a simple and rapid staining procedure, may suggest that 10% concentration of the 3rd type of staining solutions is efficient for identification of *H.pylori* organisms in the gastric tissue

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Figure (2): Photomicrograph of gastric tissue stained with H&E. The arrow indicates the appearance of ***Rpylori*** organisms stained with pink color (x 1000).

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