

## Technical report

# Modified Genta triple stain for identifying *Helicobacter pylori*

Hala M T El-Zimaity, Jian Wu, David Y Graham

### Abstract

**Aim**—To evaluate whether lead nitrate could replace uranyl nitrate in the Genta stain for *H pylori* without sacrificing the advantages of the triple stain (Steiner silver impregnation combined with Alcian blue and haematoxylin/eosin (H&E)).

**Methods**—A comparison was made in 16 specimens between the original triple stain and the revised version. One pathologist evaluated all sections.

**Results**—Direct substitution of lead nitrate for uranium nitrate produced well stained organisms without interfering with H&E or Alcian blue staining. No difference was found in the ability to identify bacteria in 11 cases with *H pylori* density of 1 or 2 (on a scale of 0 to 5).

**Conclusions**—The potential chemical and radiological hazards associated with uranium nitrate can be eliminated by using lead nitrate without sacrificing the advantages obtained by using the triple stain.

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Keywords: *Helicobacter pylori*; Genta stain; uranyl nitrate; lead nitrate

The Genta stain<sup>1</sup> is used routinely in our laboratory because it allows simultaneous visualisation of the bacteria, the histological features of gastritis, and any other pathology such as intestinal metaplasia. The Genta stain has potential disadvantages which include the requirement for uranyl nitrate which, in some countries, may be difficult or impossible to obtain. Because of the complexity, time involved, and the potential radiological hazard<sup>2</sup> of uranyl nitrate, many laboratories have shied away from using the Genta stain. In this communication, we evaluated whether the widely available lead nitrate could replace uranyl nitrate in the Genta stain without sacrificing the advantages of the triple stain.

### Methods

#### MODIFIED TRIPLE STAIN

#### Reagents

1. Gum mastic (Polyscientific), 2.5%; refrigerator at 4°C.
2. Lead nitrate-gum mastic solution: mix 0.5 g lead nitrate, 40 ml 70% alcohol, and 10 ml of 2.5% gum mastic. May be reused, but discard after 2 months. Refrigerate at 4°C.

3. Silver nitrate, 1%: 0.5 g silver nitrate in 50 ml distilled water. Make fresh each time and filter before use.
4. Silver nitrate, 0.04%: 0.04 g silver nitrate in 100 ml of distilled water. Make fresh each time.
5. Hydroquinone, 2%: 5 g hydroquinone in 250 ml distilled water. Make fresh each time.
6. Reducing solution: mix 100 ml of 2.5% gum mastic, 250 ml of 2% hydroquinone, and 50 ml absolute alcohol. Make just before use, filter through Whatman No 4 filter paper, and add 25 ml of 0.04% silver nitrate. Do not filter after adding the silver nitrate. This solution will have a milky appearance when the gum mastic is added.
7. Alcian blue solution, 1% in acetic acid, pH 2.5 (Polyscientific).
8. Haematoxylin (Gill, triple strength) (Stat-Lab).
9. Treosin (StatLab).

#### Procedure

Before staining, place a plastic Coplin jar in a 45–60°C water bath to heat. Prepare the reducing solution and place in the preheated Coplin jar. Steps 1 and 8–18 can be easily performed on an autostainer.

To check the accuracy of the modified Genta stain for identifying *H pylori*, a comparison was made in 16 specimens between the original and the revised version. One pathologist (HE-Z) evaluated all sections.

1. Deparaffinise and hydrate to distilled water.
2. Sensitise sections by placing in room temperature lead nitrate-gum mastic solution and microwave at high power for 30 s.
3. Allow slides to remain in solution for another 30 s (gently stir with a glass rod).
4. Rinse in distilled water.
5. Place sections in 1% silver nitrate, microwave but do not boil (2 min 15 s).
6. Allow slides to remain in the warm solution for 90 s.
7. Wash in distilled water (×4).
8. Place in a reducing solution and microwave at high power for 30 s.
9. Allow slides to remain in the warm solution for 3 min or until properly developed.
10. Rinse in distilled water (×4).
11. Hydrate back to distilled water.
12. Place slides in Alcian blue (10 min).

Gastrointestinal  
Mucosa Pathology  
Laboratory, Veterans  
Affairs Medical Center  
and Baylor College of  
Medicine, 2002  
Holcombe Blvd,  
Houston, Texas 77030,  
USA  
H M T El-Zimaity

Department of  
Medicine, Veterans  
Affairs Medical Center  
and Baylor College of  
Medicine  
J Wu  
D Y Graham

Correspondence to:  
Dr El-Zimaity.

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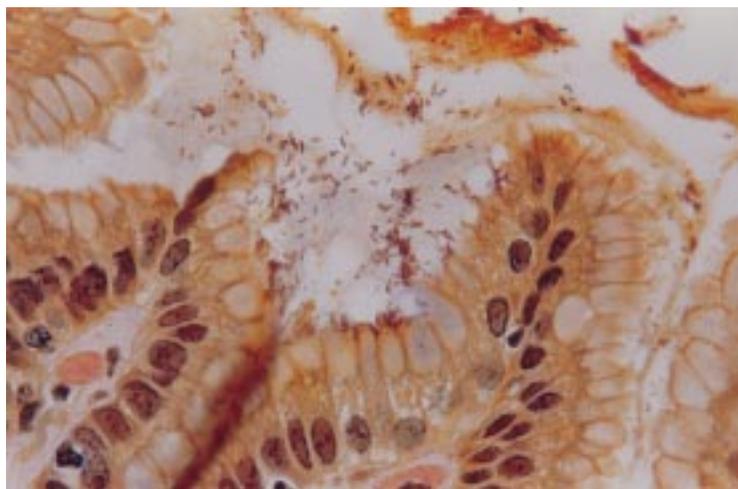


Figure 1 Gastric mucosa showing *H pylori* using the modified Genta stain.

13. Rinse in distilled water.
14. Stain in Gill's haematoxylin (5–8 min).
15. Rinse in tap water.
16. Quickly dip in ammonia water or lithium carbonate (0.5%).
17. Wash in tap water (two changes).
18. Stain in eosin (five min).
19. Wash in tap water.
20. Dehydrate through graded alcohols and clear in xylene.

### Results

Direct substitution of lead nitrate for uranium nitrate produced well stained organisms without interfering with haematoxylin and eosin or Alcian blue staining (fig 1). Eleven cases with *H pylori* density of 1 or 2 (on a scale of 0 to 5)<sup>3</sup> were examined. No difference was found in the ability to identify the bacteria. The staining time was also reduced by 46 minutes by the eliminating the gum mastic step between silver nitrate impregnation and reduction and by using the microwave oven at sensitisation, silver impregnation, and in the reduction step. Because of the variation in microwave wattage, timing in the microwave will vary. Generally, 2 minutes 15 seconds for silver impregnation and 30 seconds

for sensitisation and reduction are adequate. A comparison between the original and the revised version is summarised in table 1.

### Discussion

The Genta stain<sup>1</sup> combined the modified Steiner method<sup>4</sup> with Alcian blue and haematoxylin and eosin to facilitate the examination of *H pylori* and its associated pathology such as gastritis and intestinal metaplasia on the same slide. While the stain is used routinely in our laboratory, it did not gain acceptance in many laboratories owing to the lengthy staining time and the use of uranyl nitrate with its potential radioactive hazards.

Steiner's method requires the use of heat to impregnate tissue with silver nitrate. Subsequently, slides are placed in a reducing solution that converts silver nitrate to black metallic silver deposits on the bacteria. Garvey and colleagues<sup>4</sup> reduced the staining time with a developing solution that was easier to prepare than the original Steiner formula.<sup>5</sup> Swisher<sup>6</sup> used the microwave oven at sensitisation and silver impregnation. Finally, Churukian further reduced the staining time by also performing the reduction step in the microwave oven rather than in a hot water bath (Churukian C, personal communication). In 1979, Elias *et al* reported that it was possible to use lead nitrate as a substitute for uranyl nitrate in the Steiner stain and thus avoid the potential radiation hazards of uranyl nitrate.<sup>7</sup> We incorporated these different suggestions<sup>4 6 7</sup> in the current modification of the Genta triple stain. The use of lead nitrate–gum mastic solution and the microwave oven at the sensitisation, silver impregnation, and reduction steps reduced the staining time by 264%. In addition, deparaffinisation (step 1) and all steps following reduction (step 9) can be done with the help of an autostainer. Lead nitrate provides a substitute that is widely available, free of any potential radiation hazard, and without loss of staining specificity. The technique is thus quicker than before, simple to perform, and possible to undertake in any laboratory. Using an autostainer, the total technical time is only 9–10 minutes.

Table 1 Comparison between the original and the revised version of the Genta stain

Procedure	Original Genta	Modified Genta
Deparaffinise and hydrate	20 s	20 s†
Sensitise sections	3 min	30 s
Slides remain in solution		30 s
Rinse in distilled water	5 s	5 s
1% silver nitrate	2 min, 15 s	2 min, 15 s
Slides remain in solution	10 min	1 min, 30 s
Wash in distilled water	5 s	5 s
Rinse in 95% and 100% alcohol (×2 each)	10 s	Eliminated
Gum mastic	10 min	Eliminated
Reducing solution	25 min	30 s
Slides remain in solution		3 min
Rinse in distilled water	5 s	5 s†
Hydrate back to distilled water	10 s	10 s†
Place slides in Alcian blue	10 min	10 min†
Rinse in distilled water	5 s	5 s†
Gill's haematoxylin	7 min	7 min†
Rinse in tap water	5 s	5 s†
Ammonia water	2 s	2 s†
Tap water	2 s	2 s†
Eosin	5 min	5 min†
Wash in tap water	2 s	2 s†
Dehydrate and clear in xylene	10 s	10 s†
<b>Total time</b>	<b>74 min, 8 s</b>	<b>28 min, 4 s</b>

†Steps that can be performed on an autostainer.

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