Letters to the Editor

Long term freeze storage of *Campylobacter pyloridis*

Since the first descriptions of *Campylobacter pyloridis* by Warren¹ and Marshall,² a rapidly growing number of articles have been published on the subject. Some of these have been summarised in recent reviews.^{3,4}

C. Pyloridis is a very fastidious organism that requires high humidity and a mixture of carbon dioxide, hydrogen, and nitrogen in the atmosphere for optimal isolation.⁵ Once isolated, frequent subculturing on fresh media is necessary to keep the strains viable. Leaving them out on the bench in room air for just a few hours can kill them. They are also extremely sensitive to freezing, and we have found that they do not survive when frozen in regular freezing media. The same observation has been made by other investigators. One method for long term storage is lyophilising without freezing, which is a cumbersome method for every day usage (Goodwin CS, Cave D, personal communication⁵).

We explored a large number of different freezing media, looking for a more suitable storage system. Various combinations of glycerol, brain-heart infusion, trypticase-soy broth, and heat inactivated serum all failed to sustain viability for more than a few weeks at -70°C . Two suitable candidates for long term storage were found, however—skim milk or whole defibrinated horse blood. Using these media, *C. pyloridis* has now been kept viable in our laboratory for as long as six months at -70°C . To compare the two methods we performed colony counts after thawing.

For this purpose reference strain NCTC 11638 was used. A 1:0 McFarland suspension in trypticase-soy broth was made, and 20 μl added to phials containing 1 ml skim milk, 1 ml defibrinated horse blood, and 1 ml equal mixture of the two.

After six months the whole blood media still contained 4×10^6 viable organisms/ml and the skim milk about 20% of that figure. Combining the two did not confer any additional advantage. Neither did adding 1% IsoVital to the media. Some of our vials were thawed and refrozen once a month during this period for comparison and had colony counts similar to those which were only thawed once, indicating that the thawing and freezing process might be of lesser importance to loss of viability than the length of the storage period.

Of the two media, we prefer freezing in whole defibrinated horse blood, since reviving the organism can be done on regular chocolate media without antibiotics. The skim milk is never 100% sterile after pasteurisation, and unless a selective media was used, overgrowth of contaminants was not uncommon.

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References

- Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273.
- Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273-5.
- Marshall BJ. *Campylobacter pyloridis* and gastritis. *J Infect Dis* 1986;153:650-7.
- Goodwin CS, Armstrong JA, Marshall BJ. *Campylobacter pyloridis*, gastritis, and peptic ulceration. *J Clin Pathol* 1986;39:353-65.
- Goodwin CS, Blincow ED, Warren JR, et al. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J Clin Pathol* 1985;38:1127-31.
- Thomas JM, Poynter D, Gooding C, et al. Gastric spiral bacteria. *Lancet* 1984;ii:100.

Cresyl fast violet staining method for campylobacter like organisms

We publicise a simple method for staining campylobacter like organisms (CLO) in gastric biopsy specimens. We have used this method with complete success for several years,¹ and it has been adopted by several other laboratories without difficulty.

The presence of CLO in the stomach was first described by Warren and Marshall in 1983,² and their report generated considerable interest. The organisms are difficult to see on haematoxylin and eosin sections, and most investigators have used a silver impregnation technique (Warthin-Starry) to stain them. We have found this technique, like most silver methods, cumbersome and sometimes fickle, especially for routine use, and so we experimented with other methods. For some time now we have been using a cresyl

fast violet method to stain CLO. The method is a simple adaptation of two standard methods,^{3,4} and it has been found to be both simple and reproducible by all of the several laboratories in which it is now being used. The organisms have a strong affinity for this stain; their characteristic morphology is clearly shown; and even very small numbers can be identified with confidence. Occasionally, some confusion can arise if there is extensive intestinal metaplasia in the stomach because the intestinal mucins produced also take up the stain, but once appreciated this ceases to be much of a problem.

CRESYL FAST VIOLET ACETATE METHOD
Fixation requires 10% formol saline, or formol sublimate (primary or secondary). Sections are paraffin or resin. Method is as follows:

- 1 Take section to water
- 2 Stain in 0.2%. Cresyl fast violet acetate for five minutes
- 3 Rinse in water
- 4 Rinse in 95% alcohol
- 5 Differentiate in Cresyl violet differentiator (95% alcohol 90 ml; chloroform 10 ml; acetic acid three drops)—nuclei violet, cytoplasm almost colourless, CLO deep blue-violet
- 6 Rinse well in absolute alcohol
- 7 Clear and mount.

CLO now seem to be firmly implicated in the pathogenesis of some types of gastritis and their identification is becoming part of the routine assessment of gastric biopsy specimens.⁵ This Cresyl fast violet method is simple and reliable and very suitable for routine use.

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References

- Burnett RA, Forrest JAH, Girdwood RWA, Fricker CR. Campylobacter-like organisms in the stomachs of patients and healthy individuals. *Lancet* 1984;i:1349.
- Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273-5.
- Moore KL, Barr ML. Smears from the oral mucosa in the detection of chromosomal sex. *Lancet* 1955;ii:57.
- Culling CFA. *Handbook of histopathological and histochemical techniques*. 3rd ed. London: Butterworths, 1974:446-7.
- Marshall BJ. Campylobacter and gastritis. *GJ Futures* 1986;1(3):4-6.