Acridine orange stain in the histological identification of Helicobacter pylori

The recent paper by Rotimi and colleagues does not mention the acridine orange stain when comparing staining methods for the identification of Helicobacter pylori. The acridine orange stain uses ultraviolet fluorescence in the identification of bacteria. The typical curved morphology of H pylori can easily be differentiated from other bacteria.

I have used this quick, cheap, and reliable stain in routine histopathology reporting for over 16 years and it has proved to be extremely useful in the identification of H pylori. Immunohistochemistry is now recognised as the “gold standard” because it is a highly sensitive and specific staining method. After the publication of the above mentioned article, 20 consecutive gastric biopsies that were positive for H pylori using the acridine orange stain were also stained using the polyclonal anti-H pylori antibody (Dako, Elly, Cambridgeshire, UK) at a dilution of 1/100. Twenty negative control cases were similarly studied. All 20 cases that were positive with the acridine orange stain were also positive by immunohistochemistry and all negative cases were also negative by immunohistochemistry.

This small study clearly shows that ultraviolet fluorescence of H pylori using the acridine orange stain is highly sensitive and compares equally with the gold standard of immunohistochemistry. The acridine orange stain may not be specific, but the morphology of H pylori is clearly visible down to the single organism (fig 1).

The only disadvantage of the acridine orange stain is that the microscopic needs a fluorescent attachment, which in my laboratory means turning the lever on a Leitz Diaplan microscope to the required position, without the need for a dark room.

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Figure 1 Helicobacter pylori stained with acridine orange. Original magnification, ×250.

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Place a few drops of reticulin solution in a beaker and add small drops of your test solution. If the test solution is formalin, the reticulin solution will turn black. A similar effect can be produced by adding the test solution to Schiff's solution. In this case, adding drops of formalin will turn the combination a deep magenta colour. The addition of a test solution of saline (the most frequently encountered alternative) will produce no colour change to Schiff's solution and will turn reticulin solution white. Because all laboratories will have both reagents already prepared on their shelves, the test may be done in a matter of seconds.

Good old fashioned chemistry to the rescue!

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ASD chloroacetate esterase. On flow cytometry, the blasts were found to be CD34 positive with expression to some extent of CD13, CD33, CD45, CD45RA, CD117 (c-kit gene product), and CD56. No expression of CD10, CD19, CD45RO or CD90 could be demonstrated. Immunohistochemical studies performed on paraffin wax embedded sections demonstrated CD34 and CD45 positivity in the blasts (fig 2A). However, these cells were MPO, CD3, and CD20 negative. CD68 expression was variable. To exclude with certainty the possibility of an unnoticeable bone marrow infiltration by the underlying breast carcinoma, complementary anti-CK stains using KL-1 and CAM 5.2 antibodies were performed. Surprisingly, the blasts showed a strong perinuclear or punctuate (dot-like) staining pattern (fig 2B). However, these cells did not react with the anti-CK19 antibody, further demonstrating the absence of bone marrow infiltration by the breast carcinoma.

CK expression by myeloid blasts is a very uncommon finding—only three publications (two case reports and one in vitro study) dealing with this matter have been published so far. In this setting, the comparison between our findings and those described in these reports allows us to make some interesting comments. First, similar to CK detection reported in various lymphomatous or plasma cell disorders, the immunohistochemical pattern of CK expression in myeloid blasts is also dot-like or perinuclear. Another similarity between these studies and our case is the use of antibodies that recognise a wide spectrum of CKs (prekeratin, KL-1, AE1/AE3, cocktail, or CAM 5.2). Indeed, the KL-1 antibody reacts with the following CK polypeptides: CK1, CK2, CK5, CK6, CK7, CK8, CK11, CK14, CK16, CK17, and CK18. The antibody cocktail AE1/AE3 recognises numerous acidic and basic keratins, namely: CK10, CK14, CK15, CK16, CK19 and CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, respectively. Finally, CK8 and CK18 are labelled by the CAM 5.2 antibody. In this regard, it is worth noting that these blasts are stained by AE1 but not by AE3 when these antibodies are used separately. Another point of interest is the similar differentiation that characterises CK expressing blasts. Indeed, the two reported cases of CK positive acute myeloid leukaemia (AML) reported belonging to the category of AML FAB M4.25 Although precise subtyping of AML arising from MDS may be difficult, the morphological, cytotoxic, and phenotypic features seen in our patient are also consistent with a myelomonocytic differentiation. Like Turner and Milliken,24 we found no CK19 expression by the blasts. Therefore, this observation shows that CK19 is a more specific marker of carcinomas showing glandular differentiation than are various pan-CK immunostains. Whatever the precise explanation for this unusual phenomenon may be, in addition to the two cases published previously, our observation illustrates that CK expression can be seen, albeit rarely, in AML.

The Vesalius Foundation supported this study (thanks to a grant from “La Loterie Nationale”).


Audit of colposcopy biopsy sectioning

In April 1999, the guidance document “Histopathology reporting in cervical screening” was issued.1 On page 45 of that document is the statement “As the appearance of the tissues, even in small biopsies, often shows considerable variation, several levels are required to ensure that small foci of disease are identified.”2 No more specific guidance was given in the document. The concept of “several levels” was discussed at the Symposium on Gynaecological Pathology held by the British Division of the International Academy of Pathology in Sheffield on 10 September 1999. It was clear from the discussion that there were many varying practices being used. The practice in

Figure 1 Bone marrow trephine biopsy. The picture shows a hypercellular bone marrow featuring dysplastic megakaryocytes, abnormal erythropoiesis, and increased numbers of blasts. These are often clustered.

Figure 2 (A) Immunostaining with an antibody directed against CD34 and (B) against CAM5.2. The stained cells look very similar. Note the dot-like staining pattern of CAM 5.2.
the laboratory at the City Hospital Nottingham was to examine two sections at each of the three levels of the material, all mounted on one glass slide. It was decided that this practice should be audited against examining two sections at each of six levels to see whether important diagnostic features were being missed by using the existing practice.

The subsequent consecutive 100 colposcopic biopsies were processed according to the standard operating procedure in the laboratory and then biopsies 2 µm thick were cut at each of six levels through the material. The levelling was rigorously controlled at 50 µm between each level. The levels were mounted as sections one to three on one slide and sections four to six on a second slide. The samples were examined microscopically (all by JF). The slide with levels one to three was examined and the diagnostic features recorded. Only then was the slide with the levels four to six examined. Any variance from the features seen in the first three levels was recorded and commented upon on the record sheet for the audit.

In only seven cases of the 100 examined was further information obtained from the second three levels (levels four to six). In four of these, the comment after examining the first three levels was that levels four to six would have been requested to be cut (always an option) because the diagnosis was not clear on the first three levels and it was felt that further sections might help to clarify the picture. A further three levels would have been requested on six cases; these four were included in the six. In the other two cases no further information was available in the extra three levels. Of the other three cases where additional information was obtained from the second three levels, two showed koilocytes in the squamous epithelium, which were not visible in the first three. This is not a clinically important finding because the management of the women would not have been affected. In the third case, the second three levels revealed focal stromal inflammation, not visible in the first three levels, also not clinically important.

Relevant histological features are almost always visible on the examination of sections from each of three levels cut from small colposcopic biopsies. In the small number of cases where the diagnosis is not clear on the first three levels, examination of the next three levels (in four of six such cases in our series) may help the pathologist to make the diagnosis. In none of our 100 cases was relevant diagnostic material missed by examining the material at three rather than at six levels. The conclusion reached has been to continue our original practice, thereby preventing the use of extra sectioning time and of twice the number of slides for each case. Critical evaluation of one versus two sections from each level was not conducted but one section would save very little cutting time and six sections sit easily on one slide.

During the course of our study, a letter appeared in the *Journal of Clinical Pathology* stating that sections at levels through the tissue should not be mounted on the same slide because the histological material may not be covered by the coverslip, or might be obscured by mountant. This was not the case on any of our 200 slides. With careful placing of the material on the slide by the section cutter (fig 1) and an automated coverslipping machine the artefacts mentioned in that letter are not encountered.

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