specificity in an ANCA positive specimen, and hence whether a diagnosis of Wegener's granulomatosis or microscopic polyarteritis is more likely. Serum samples associated with cytoplasmic fluorescence (cANCA) and anti-proteinase 3 antibodies are found in nearly 90% of all patients with active generalised Wegener's granulomatosis.³ Perinuclear fluorescence (pANCA) with antimyeloperoxidase antibody activity is present in about 80% of patients with active microscopic polyarteritis. These specificities occur in almost no other disease. However, pANCA and other neutrophil cytoplasmic fluorescence patterns ("atypical" ANCA) with activity against elastase, lactoferrin, lysozyme, cathepsin G, and some unidentified antigens (reports of β -glucuronidase have not been confirmed) have been described in rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, primary sclerosing cholangitis, and occasionally in other conditions. These antibodies do not indicate the presence of a vasculitis and do not correlate with disease activity. They have no clinical significance.

Finally, ELISAs indicate the level of ANCA antibody activity better than titration with IIF. Antibody levels correlate well with disease activity in both Wegener's granulomatosis and microscopic polyarteritis. In our experience, all patients presenting for the first time with these conditions have high titres of the corresponding antibodies. Levels usually fall with treatment, and can be used to monitor the response to treatment.

It is possible for a laboratory to make its own ELISA plates for antiproteinase 3 and antimyeloperoxidase antibodies. Each antigen is available commercially in a purified form. We coat plastic microtitre plates with these antigens at a concentration of about 0.5 mcg/ml in phosphate buffered saline at 4°C for 18 hours, and then block the plates with 1% human serum albumin (HSA) (Sigma) for one hour. It is possible to coat a second round of plates with the same antigen preparation after it has been removed from the wells. Serum samples are tested at a 1 in 50 dilution, and the binding is determined by subtracting that seen on a plate coated with just HSA. This technique overcomes the non-specific binding that occurs with some infections. The inclusion of standard borderline and positive serum samples allows values to be compared between assays. Control serum samples are available commercially, but otherwise there are no international units or any way of comparing binding. We report results as negative, borderline, low, medium, or high titres, because these are easier to interpret intuitively than numerical values. There is a need for a quality control programme to standardise results between laboratories. Kits for assays for antiproteinase 3 and antimyeloperoxidase antibodies are also available commercially.

There are several additional comments that I would like to make about testing for ANCA as described in the Broadsheet.

Serum samples can be screened on ethanol fixed, normal peripheral blood smears rather than using purified neutrophil preparations. ANCA were first described using peripheral blood films, and these are cheap and convenient. However, it is usually only possible to examine two samples per slide and there may be some background fluorescence. Commercial slides with six or 12 wells per slide are also available but are expensive.

The Broadsheet describes ANCA in classic polyarteritis but this is not correct. pANCA are not found in classic polyarteritis unless microscopic polyarteritis is also present. Classic polyarteritis, or polyarteritis nodosa involves medium vessels only, is characterised

by "aneurysms" of the coronary, coeliac, mesenteric, and renal arteries, and ischaemia in these organs. It is an uncommon condition, and it not associated with ANCA. However, probably half of all patients with microscopic polyarteritis have features of classic polyarteritis too. It is these patients who have pANCA with antimyeloperoxidase specificity.

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Note: I am submitting a similar letter to *Pathology*, the journal of Australian pathologists.

Dr Lock comments:

I read with interest the comments of Dr Savage regarding the ACP Broadsheet on the detection of ANCA. Several points were raised and I should like to address them in the order they occurred. Dr Savage contends that "most laboratories ... confirm positive serum samples ... using ELISAs". While this may be true in mainland Europe and, presumably in Australia, in the UK only one third of the laboratories registered with UK NEQAS use ELISAs. A slightly higher proportion, just over 40%, use formalin fixation. That said, however, it is our practice to further subtype all fluorescence positive results by ELISA for proteinase 3 and myeloperoxidase antibodies.

I would agree that proteinase 3 antibodies have a high specificity for a diagnosis of Wegener's and myeloperoxidase antibodies a high specificity for microscopic polyarteritis. However, I feel that the statement that these specificities "occur in almost no other disease" is very broad. False positive results do occur. For example, myeloperoxidase antibodies may be found in systemic lupus erythematosus and Henoch Schönlein purpura¹ and in rheumatoid arthritis.² Furthermore, proteinase 3 and myeloperoxidase antibodies are the major reactivities in necrotising vasculitis, but are not the only reactivities. Anti-elastase and anti h-lamp-2 have been described.

To dismiss atypical patterns as being of no clinical significance is perhaps ungenerous. I agree that they have poor specificity, but are supportive data in the right clinical background—for example, in ulcerative colitis. Furthermore, we have found that the titre of IgA class ANCA does correlate with disease activity in childhood Henoch Schönlein purpura.

Whether ELISAs are better indicators of disease activity remains to be confirmed. Certainly fluorescence titres have not been a universal success, but a recent case study suggests similar problems will be found with ELISA.³ I strongly support the sentiments of Dr Savage with respect to the need for international standards for these assays.

We have no experience in the use of peripheral blood smears as a source of antigen. However, we have examined a number of commercial slides and the preparations are very variable. In many cases the cell morphology is not well preserved and defining the patterns of ANCA is difficult.

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Physical state of human papillomavirus using non-isotopic in situ hybridisation

It was interesting to note the application of non-isotopic in situ hybridisation (NISH) for the detection of the physical state of human papillomavirus (HPV) in cervical intra-epithelial neoplasia (CIN) in the study by Pöllänen *et al.*¹ However, there appears to be a discrepancy in the interpretation of the NISH signal patterns in the results section of the abstract. This was rather surprising as the correct interpretation is in fact quoted in the text of the last paragraph of the methods section (although the reference number is incorrect). NISH signal patterns may either be diffuse (episomal, type 1), punctate (integrated, type 2), or punctate and diffuse, representing both episomal and integrated virus in the same cell/tumour (type 3).²

In addition, there is also a discrepancy between the abstract and text regarding the number of cases demonstrating a type 3 NISH signal. Nevertheless, it is far less than that reported previously, where half of the archival lesions defined morphologically as CIN III with morphological evidence of HPV in patients from the United Kingdom and South Africa contained either a type 2 or type 3 NISH signal.³ This discrepancy could be attributed to a smaller number of cases in the Finnish study where six of eight cases with CIN III (and morphological evidence of HPV infection) harboured HPV with a type 1 pattern (episomal).¹ The alternative explanation for not detecting integrated HPV using NISH may be technical; nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate as a substrate in the detection system tends to diffuse within the nucleus and not impart a crisp clear punctate signal (type 2) as is demonstrated with peroxidase/aminomethylcarbazole. (Cooper, Herrington and McGee, personal observations). This is crucial if NISH is to be used as a method to detect the physical state of HPV, as caution is required regarding both technique and interpretation.

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Comparison of cyanmethaemoglobin method of estimating haemoglobin concentration using four different analysers

Parameter	Analyser			
	Sysmex NE8000	Sysmex NE8000 (1 in 5 dilution)	Coulter S880	Cobas Argos
Haemoglobin (g/dl)	12.9	11	10.4	10.5
HCT	0.329	0.31	0.323	0.325
MCV (fl)	101.5	NA	100	99
MCH (pg)	39.8	35.5	32.2	32.2
MCHC (%)	39.2	36.1	32.2	32.4
WCC ($\times 10^9/l$)	6.45	NA	7.6	8
Patient's sample ($\times 10^9/l$)	166	NA	181	182

HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; WCC = white cell count.

Inaccurate haemoglobin estimation in Waldenström's macroglobulinaemia

We read with interest the article by Goodrick *et al.*¹ A similar problem was observed in a patient with Waldenström's macroglobulinaemia using the Sysmex NE 8000 automated blood analyser when the patient's plasma haemoglobin concentration was overestimated. The blood sample repeatedly failed the critical limits for mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. A 1 in 5 dilution of the sample in Sysmex diluent partially corrected the problem.

The Sysmex NE 8000 converts haemoglobin to a sulphate derivative using sodium lauryl sulphate.² Other analysers using the cyanmethaemoglobin method of estimating haemoglobin concentration gave satisfactory results (table).

The Sysmex diluent PK-30L is described as an isotonic solution containing boric acid, sodium tetraborate, dipotassium EDTA, and sodium chloride. Whole blood (6 μ l) is diluted with 2.0 ml diluent before mixing with 1.0 ml Sulfolysers 220A, a lysing solution containing 0.17% sodium lauryl sulphate. The haemoglobin sulphate is measured at 534 nm within two minutes of sampling. We have used the patient's plasma (3.6 μ l) and measured the absorbance at 534 nm after mixing with diluent and Sulfolysers. There was an increase in optical density (OD) of 0.07 units on addition of the lysing agent. This could be abolished by pretreating the plasma with 2-mercaptoethanol. Substitution of normal saline as diluent or substitution of a 0.17% sodium lauryl sulphate solution in water as lysing agent also prevented the rise in OD. Plasma from a patient with IgG myeloma and plasma from a normal donor did not exhibit this phenomenon.

We conclude that pentameric IgM in the patient's plasma is barely soluble in PK-30L diluent. Addition of Sulfolysers decreases the solubility of the paraprotein and the time taken to redissolve is longer than the analysis time of the instrument. We suggest that individual IgM paraproteins may present problems with haemoglobin measurement depending on the instrument and the measurement method used. The falsely elevated haemoglobin concentration in our patient was not sufficient to be obvious clinically.

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Pitfalls in diagnosing coeliac disease

We were interested to read the recent paper by Shidrawi *et al.*¹ which confirms our experience in a central hospital. Coeliac disease is commonly identified in northern Portugal² and recently we reviewed 25 children referred to our unit over a period of eight years with a provisional unconfirmed diagnosis of coeliac disease.³ All of these patients failed to relapse on postchallenge small bowel biopsy after at least nine months on a normal diet.

To identify the probable cause for the "false" diagnosis of coeliac disease we analysed the diagnostic criteria and reviewed the histology of all previous biopsy specimens. The pathologist, experienced in paediatric intestinal histology (FC), was unaware of clinical details of each patient.

Results showed that mean age of presentation was 8.6 months and symptoms were typical of coeliac disease, mainly diarrhoea and a failure to thrive. Seven (28%) patients had been placed on a gluten-free diet based on clinical suspicion without small bowel biopsy. Of the remaining 18 children who underwent prediet biopsy, histological reassessment showed that three had been inadequately processed leading to difficult interpretation, five had minor abnormalities including partial villous atrophy, six had "flat mucosa", and four were inappropriately reported as having subtotal villous atrophy.

A definite diagnosis of coeliac disease in young children requires three intestinal biopsies.⁴ In older children a simpler diagnostic approach may be acceptable in some circumstances.⁵ Despite wide medical awareness of the need for an intestinal biopsy before placing patients on a gluten-free diet it is still common to see patients placed on the diet without undergoing biopsy, leading to unnecessary strain on the patients and their families, and makes it difficult to decide when to stop taking postchallenge biopsy specimens, and may divert attention from the correct diagnosis. Our results also stress other aspects: (a) small intestinal biopsy specimens need to be handled very carefully, and preferably should be assessed first through a dissecting microscope; and (b) special expertise in evaluating intestinal biopsy specimens is important in order to reduce the risk of misinterpreting artefacts and minor abnormalities.

Six children with true villous atrophy at onset did not relapse and have been symptom-free on a normal diet; transient gluten intolerance must be considered as a likely diagnosis in these patients although strict criteria were not adhered to.⁶ Despite scepticism about this entity, no other factors could be identified and the gluten-free diet alone led to marked improvement in these patients. In summary, our data reinforce the conclusions of Shidrawi *et al.*¹ and stress the relevance and need for accurate interpretation of histology in the diagnosis of coeliac disease.

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Book reviews

The Endometrium. A Clinicopathologic Approach. DS Heller. (Pp 224; £68) Igaku-Shoin. 1994. ISBN 0-89640-244-4.

Coming from a background in gynaecology and pathology, the author aims to improve communication between the specialties by presenting short sections on both subjects. She has missed her opportunity, however, by dividing the book into separate pathology and gynaecology sections rather than discussing them together in a clinicopathological context.

Both parts are too short and do not include much information that would be useful to anyone other than a beginner in their own specialty and contain too much that is irrelevant to someone from another discipline. For example, neither gynaecologists nor pathologists need a picture of a microtome, which in any case is rotated through 90°. There are many excellent illustrations but some are wrong, covered in other chapters or contradicted by others elsewhere in the book. If the pathology section is aimed at gynaecologists, then it is too detailed but there is insufficient detail for it to be used as a bench book in histopathology. Many of the references are to standard texts, such as *Biopsy Pathology of the Endometrium* by Buckley and Fox (which is a better book), and references to original papers are out of date: nothing