4 Morgan PR, Shirlaw PJ, Johnson NW, Leigh IM, Lane EB. Potential applicaions of antikeratin antibodies in oral diagnosis. *J Oral Pathol* 1987;16:212–2.
5 MacDonald AW, Fletcher A. Expression of cytokeratin in the epithelium of dentigerous cysts and odontogenic keratocysts: an aid to diagnosis. *J Clin Pathol* 1989;42:736–9.

Drs MacDonald and Fletcher comment:

We are grateful to Matthews and Browne for their comments and we note the differences in staining pattern in odontogenic keratocyst linings using LP34 between their method and the method we described. The methods are different in several important respects. The tissue on which we based our observations had not been subjected to any form of decalcification. We used a normal rabbit serum pretreatment and incubated the section overnight with primary antibody (LP34; 1/200 dilution). This, we have found, virtually eliminates background staining while giving strong reliable reactivity. The method described by Matthews and Browne uses tissue which has been decalcified in 10% formic acid and pretreated with ovalbumin, and the incubation period and dilution of the primary antibody were different (LP34; 1/40–1/100 for 60 minutes).

Experienced immunohistochemists agree that to expect reproducible results using monoclonal antibodies, such as LP34, the processing of the tissue and methods of staining used should be similar wherever possible.

We should like to take this opportunity to reactify a typographical error and a detail from the material and methods section of our recent article. The antibody CAM 5·2 was supplied by Becton Dickinson and the secondary antibody we used was a peroxidase conjugated rabbit antimouse serum (Dako; 1/25 dilution).

AW MACDONALD
A FLETCHER

Statistics on microcomputers

The article on correlation and regression shows the correlation coefficient of r = 0·021 for the data in figure 1c, depicting a recurvilinear relation between measurements A and B (page 5).[1]

With all due respect, I believe that the correlation coefficient is inappropriate for a recurvilinear relation of this sort; instead, an appropriate quadratic equation could be derived for the relation and the correlation calculated from that; alternatively, and more simply, the correlation ratio or eta coefficient.[2]

The eta coefficient calculated for the measurements read (admittedly with some small inaccuracy) from the graph shown in fig 1c provides a value of 0·9154. In other words there is a very good recurvilinear correlation shown, and this figure certainly fits better with the "eyeball" estimate of linear quality to the points on their graph.

The eta coefficient does not seem to be widely used. It is indeed a little crude compared with deriving the proper quadratic equation, but the approximations are very close and rarely give rise to differences larger than the second decimal place.

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1 Brown RA, Swanson Beck J. Statistics on microcomputers: A non-algebraic guide to their appropriate use in biomedial research and pathology laboratory practice 4. Correlation and regression. *J Clin Pathol* 1989;42: 4–12.
2 Downie NM, Heath RW. Other correlation techniques. In: *Basic statistical methods*. New York: Harper and Row, 1974:110–14.

Drs Brown and Swanson Beck comment:

Dr Muckle seems to have misunderstood our reason for including fig 1c in article 4 of our recent series on the use of microcomputers for statistical analysis.

In the second paragraph we emphasised that preliminary scatter plots should be used to assess the data visually and determine whether the points were obviously distributed along a straight or curved line or whether they were scattered randomly. In the third paragraph we defined the product-moment correlation coefficient and explained that this technique is invalid when there is a strong non-linear relation between the measurements: fig 1c was presented as a serious warning on such inappropriate use of correlation coefficient.

When faced with data such as those given in fig 1c, it would be usual to attempt to fit a curve as suggested by Dr Muckle, but this was beyond the scope of the introductory series of articles. Both Minitab and Statgraphics can be used to fit a curve to data and to calculate r^2 as a measure of the amount of the variability explained by the curve. The correlation ratio to which Dr Muckle refers is synonymous with r^2: when calculated for fitting a quadratic function to the data and it was greater than 0·9. It is, however, important to remember that this expression uses the term "correlation" loosely because it properly refers to "the strength of the linear relationship between two or more variables" (Kotz S & Johnson NL, 1982 *Encyclopedia of Statistics* Vol 2, p 193).

C-myc oncogene product p62 in ovarian mucinous neoplasms

In the article by Polacarz *et al* the oncogene protein product of *c-myc* was noted to differ in localisation in ovarian mucinous cystadenoma and cystadenocarcinoma. Such a difference can be explained by either compartmentalisation or artefactual localisation. The latter was considered to be unlikely because such a difference could be seen between two different epithelial types on the same tissue block in studies on colonic polyps.

Such a conclusion is based on the assumption that the *c-myc* protein in different epithelia reacts similarly with fixatives and that the nuclear structure (especially the nuclear matrix), with respect to its ability to retain the *c-myc* protein, is in a similar state in both epithelia.

The first assumption is not always true. Abnormal *c-myc* protein is not unknown in malignancies (in Raji cell line) and these proteins in different structures may behave differently when exposed to fixatives. This has been noted in mutated *c-myc* in vitro. Lee *et al* defined the regions in *c-myc* protein which are important but not necessary for nuclear localisation. In transfected cells expressing different mutants of *c-myc*, however, there is a discrepancy in the localisation of the protein when deletions of these regions occur in the mutant cells, depending on the method of processing.

If the protein is similarly mutated in malignant epithelium we might be seeing an artefact of protein displacement during tissue processing, but structural changes in protein are probably uncommon in malignancies. In fact, there is evidence to suggest that preservation of specific regions of the *c-myc* protein is important for its transforming ability and that these regions overlap with those which are most important for nuclear localisation.[1] Whether there are contributory factors which may cause protein displacement during processing, such as structural changes in the nuclear matrix and the chromatin state, need to be investigated further.[2]

Such an analysis does not exclude a change in compartmentalisation nor does it negate the importance of consistently finding cytoplasmic localisation of *c-myc* in certain neoplastic states. After all, what histology demands is only a reproducible artefact. On the other hand, there would be important implications for the function of the *c-myc* protein if we assume that its real in vivo localisation is affected by targetting in malignant epithelium. We need to assess critically tissue processing.[3,4]

We have shown previously that fixation and embedding do affect the quantitation and localisation of *c-myc* protein in cell lines and normal tissues.[3] The optimal conditions for more reliable and consistent localisation of *c-myc* protein in frozen tissue have been defined.

Purely cytoplasmic staining is very common, even in many normal tissues in paraffin wax blocks, and a complete nuclear localisation is seen in the corresponding frozen blocks if processed using the conditions we have defined. In a case of mucinous ovarian tumour of borderline malignancy, we found the same discrepancy between the paraffin wax and frozen blocks. Although it is hard for us to prove that it is the nuclear localisation that is real, it will probably be more difficult to argue that the cytoplasmic localisation is not artefactual.

For optimal immunostaining, the frozen sections are freshly cut, incubated at 45°C for five to 30 minutes, air dried for one hour and fixed in 1% paraformaldehyde for 30 minutes. Periodate-lycine-paraformaldehyde gives similar results, while acetone or formalin give weaker staining with a tendency of more cytoplasmic than nuclear staining as the duration of fixation increases. The sections are then put into 0·1%. Triton-X-100 for 10 minutes. Standard peroxidase or APAAP techniques can then be applied.

In trying out these methods we have seen different artefacts, leading us to conclude that cytoplasmic localisation is easily simulated by manipulation of the tissue processing.

Lastly, we might also need to look at the difference in nuclear matrix between benign and malignant epithelia. It is probably not too far-fetched to imagine that cytoplasmic localisation of *c-myc* protein in paraffin wax sections is related more to the chromatin pattern. If this were the case, maybe a more reasonable speculation on the function of *c-myc* is that it helps to maintain the chromatin in a state ready for cell division.

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1 Lee W, Dang CV, de Lange T, *et al.* Definition of regions in human *c-myc* involved in transformation and nuclear localisation. In: Alt FW, Harlow E, Ziff EB, eds. *Nuclear oncogenes; current communications in molecular biology.* New York: Cold Spring Habor Laboratory, 1987:179–85.
2 Evan GI, Hancock DC. Studies on the interaction of the human *c-myc* protein with cell nuclei: p62 *c-myc* as a member of a discrete subset of nuclear proteins. *Cell* 1985;**43**:253–7.
3 Loke SL, Neckers LM, Jaffe ES *et al. C-myc* protein in normal tissue—Effects of fixation on its apparent subcellular distribution. *Am J Pathol* 1988;**131**:29–37.
4 Eisenman R, Hann SR. *myc*-Encoded proteins of chicken and man. *Curr Topics Microbiol Immunol* 1984;**113**:192–7.

Drs Polacarz and Stephenson comment:

We consider the differing distribution of the *c-myc* protein p62$^{c\text{-}myc}$ in benign and malignant mucinous ovarian tumours to be of considerable importance. Of particular interest is the identification of a subset of borderline mucinous tumours that may behave aggressively, and this possibility is currently under investigation.

The authors' views concerning both mutation of p62$^{c\text{-}myc}$ in malignant neoplasms and the possible effect of fixation on subcellular distribution of the gene product are interesting and warrant further investigation. Unlike the authors, however, we have only very rarely found cytoplasmic staining in non-malignant cells and tissues which are paraffin wax embedded. This is true for normal tissues, including glandular epithelium from a variety of sites, fibroblasts, and inflammatory cells expressing the gene, and for benign neoplastic glandular epithelia. Thus in our hands cytoplasmic staining does seem to reflect a genuine perturbation of cell biology towards expression of the malignant phenotype. We thus consider the observations outlined in our paper to remain valid.

We would gladly welcome the views of other workers on this point and await further developments in this area with interest.

Dipstick urinalysis for bacteriuria

We noted the comments of Coia and Wills with interest.[1] Both they and other recent authors[2 3] seem to have assumed that significant growth on culture is the gold standard and that the dipstick is wrong if there is a discrepancy, particularly in the case of negative dipstick and positive culture. But the third and perhaps most important consideration is whether the growth has any clinical importance.

We investigated this problem last year when we examined 5834 urines for protein, blood, nitrite, leucocyte esterase and culture: 2560 (44%) were negative for all four analytes, 33 of which gave a significant growth comprising 0·6% of total specimens, but 9·0% of the 369 significant growths. These findings are similar to others.[1 3]

From the total we examined 1521 inpatient specimens in greater detail. A clinical bacteriologist visited all available patients who had a specimen with significant growth, or if this was not available, examined the clinical notes to try to determine whether the growth was clinically important. This was assessed from the history and clinical findings, especially regarding temperature, dysuria, frequency and loin or suprapubic pain. A decision could usually be made at the first visit but in a few patients repeated inquiries had to be made, especially concerning the effect of treatment on symptoms. The results are summarised in the table where positive means positive for any one of the four analytes and negative means negative for all four analytes.

Table Comparison of dipstick and significant growth with clinical importance in 1521 inpatients

	No of specimens	*Clinically important*
Significant growth + positive stick	98	51
Significant growth + negative stick	16	0
Total	114	51

The causes of this high number of significant growths with no clinical importance (63 of 114; 55%) are sometimes speculative and may vary from place to place. But in our situation, it does seem reasonable to abandon culture in specimens with negative stick results. This can be refined further. We found that the most important single dipstick result regarding a positive culture was a positive nitrite, alone, or in any combination. If nitrite was negative, then the next most important was a positive leucocyte esterase. This alone, however, was associated with an increased number of negative culture results. But if positive in the absence of nitrite positivity and in the presence of positive results in both protein and blood, then there was a closer relation between a positive dipstick result and a positive culture of clinical importance. Furthermore, if we adopted these two dipstick criteria as indications for culture: (i) positive nitrite alone or in any combination; (ii) negative nitrite but positive for leucocyte esterase blood and protein, then all of those found dipstick negative, even when yielding a sigificant growth on culture, were not found to be clinically important.

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1 Coia JE, Wills G. Dipstick urinalysis for bacteriuria. *J Clin Pathol* 1989;**42**:444.
2 Doran HM, Kensit JG. Screening for bacteriuria with Clinitec—200. *J Clin Pathol* 1988;**44**:1127–9.
3 Brown Hazel. Chemical pre-screening of urines submitted for bacteriological analysis. *Med Lab Sci* 1988;**45**:304–7.

Dr Coia comments:

In our own study we did not attempt to evaluate the clinical importance of all our culture positive isolates. The question we wished to address was how good the semiautomated dipstick test was at predicting the presence of bacteriuria. Significant growth on culture is the accepted standard method for such detection, and as such, any novel method should be compared with it. The data presented by these authors, and in the literature cited by them, would all seem to suggest that the dipstick test is inferior in this respect.

The interpretation of the clinical importance of such bacteriuria is a separate (albeit related) issue, and the point is well made by Loker *et al* that the results of all diagnostic tests must be interpreted in the light of the clinical presentation. In this context it should be remembered that the clinical importance of bacteriuria is not dependent solely on the presence of symptoms and signs, including those mentioned by the authors. It is widely acknowledged that entirely asymptomatic bacteriuria may be clinically important in certain groups which include children and pregnant women. It is not stated in this letter whether the definition of clinical importance was extended to include such groups.

Automated measurement of plasma viscosity using the Coulter Viscometer II

With reference to the recent letter from DI Fish *et al*[1] regarding the paper by Cooke and Stuart,[2] we would like the opportunity to bring the subject up to date. Having reviewed the findings, both in the report by Cooke and Stuart and the DHSS document,[3] we found that the daily shutdown procedure has been modified to incorporate a greater concentration of sodium hypochlorite solution (4% available chlorine) and that cleaning the sample valve daily has been recommended.

The instrument software has been improved to reduce the incidence of "data scatter" messages when analysing high viscosity samples, though in the event of this message still being encountered, a second analysis of the sample is recommended. Viscosity measurements greater than 5 mPa.s are now reported by the instrument, but are flagged with an asterisk to indicate that the value is outside the linear response range of the instrument. Samples with an extreme increase in plasma viscosity—for example, in severe macroglobulinaemia—will generate the message "BLOCKAGE ? OVER-RANGE ?" which would draw the operator's attention to an unusually high plasma viscosity or fibrin clot.

We believe that these modifications afford improved instrument performance and provide added benefits to the clinician.

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1 Fish DI, Jackson RF, Dawson DW. Automated measurement of plasma viscosity by capillary viscometer. *J Clin Pathol* 1989;**42**:780.
2 Cooke BM, Stuart J. Automated measurement of plasma viscosity by capillary viscometer. *J Clin Pathol* 1988;**41**:1213–16.
3 Fish DI, Jackson RF, Dawson DW. *An evaluation of the Coulter Viscometer* II. London: DHSS report STD/88/14, July 1988:1–25.

CLO in Meckel's diverticula

de Cothi *et al* recently reported the presence of Campylobacter-like organisms (CLO) in four of 13 Meckel's diverticula which contained heterotopic gastric mucosa.[1] We should like to report our experience in 29 such cases which contained heterotopic mucosa and which were examined histologically in the Departments of Histopathology at the Royal Victoria Hospital and the Belfast City Hospital.

Between 1981 and 1985, 109 diverticula removed from 63 men and 46 women were