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 curvilinear relation between measurements A and B (page 5).

With all due respect, I believe that the correlation coefficient is inappropriate for a curvilinear 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.

The eta coefficient calculated for the measurements read (admittedly with some small inaccuracy) from the graph shown in fig 1c provides a value of 0.1954. In other words there is a very good curvilinear 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 greater than the second decimal place.

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C-myc oncogene product p62 in ovarian mucinous neoplasms

In the article by Polacca et al on the oncogene protein product of c-myc was noted to differ in localisation in ovarian adenocarcinoma and adenocarcinoma. 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 tissues 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 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. 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.

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 some 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 nuclear localisation is affected by targeting in malignant epithelium. We need to assess critically tissue processing.2,3

We have shown previously that fixation and embedding do affect the quantitative and localisation of c-myc protein in cell lines and normal tissues. 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 immediately at 45°C for five to 30 minutes, air dried for one hour and fixed in 1% paraformaldehyde for 30 minutes. Periodate-lysin-paraformaldehyde gives similar results, while acetone or formalin weakens 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 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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Dipstick urinalysis for bacteriuria

We noted the comments of Coia and Wills with interest. Both they and other recent authors 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 analyses, 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.

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, change in appetite 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 the positive mean is for any one of the four analyses and negative means negative for all four analyses.

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 and protein, then all of those found dipstick negative, even if yielding a significant growth on culture, were not found to be clinically important.

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 the letter whether the definition of clinical importance was extended to include such groups.

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

<table>
<thead>
<tr>
<th>No of specimens</th>
<th>Clinically significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant growth + positive stick</td>
<td>98</td>
</tr>
<tr>
<td>Significant growth + negative stick</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
</tr>
</tbody>
</table>

Automated measurement of plasma viscosity using the Coulter Viscometer II

With reference to the recent letter from DI Fish et al regarding the paper by Cooke and Stuart, we would like the opportunity to bring the subject up to date. Having reviewed the findings, both in the reference by Cooke and Stuart and the DHSS document, we found that the daily shutdown procedure has been modified to incorporate a greater concentration of sodium hydrochloride 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.
C-myc oncogene product p62 in ovarian mucinous neoplasms.

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http://jcp.bmj.com/content/43/1/85.2.citation

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