Table *Influence of heat inactivation of human sera on results of anti-T vaginalis IgG ELISA*

Serum designation*	Absorbance†		Titre IgG	
	Unheated	Heated	Unheated	Heated
096	0·03	0·45	0	4000
103	0·00	0·30	0	4000
104	0·02	0·30	0	4000
106	0·02	0·54	0	8000
020	0·07	0·99	0	>8000
034	0·03	0·88	1000	>8000
015	0·05	0·65	2000	8000
017	0·03	0·39	2000	4000
032	0·06	1·26	1000	>8000
027	0·00	0·32	0	4000
091 T	0·29	0·74	4000	8000
105 T	0·09	0·38	1000	4000
062 T	0·03	1·52	1000	>8000
078 T	0·10	1·35	1000	>8000
066 T	0·07	0·99	2000	>8000
091 T	0·07	0·93	2000	8000
028 T	0·07	0·98	1000	>8000
034 T	0·28	1·41	4000	>8000
099 T	0·05	1·07	0	>8000
075 T	0·04	1·27	2000	>8000

*Sera designated T are from women with positive culture for *T vaginalis*.
†At a serum dilution of 1/4000.

alis antibodies by ELISA. The effect of heat inactivation on the results of ELISA was therefore investigated.

Twenty sera, 10 from women positive by culture for *T vaginalis*, and 10 from unaffected patients, were tested by ELISA as previously described[2] before and after heat inactivation (56°C, 30 minutes). The results are given in the table. The titres of all tested sera became positive after inactivation. Francis *et al* consider that the non-specific attachment of heat-aggregated immunoglobulin to the solid phase is the possible cause of increased absorbancies.[1] According to our results, heat inactivation of sera should also be avoided in anti-*T vaginalis* IgG, and possibly, when determining specific antibodies of any kind by ELISA.

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2 Gombošová A, Valent M. Use of ELISA in proof antibodies to Trichomonas vaginalis. *Bratislava lekhr Listy* 1988;**89**:689–93.

Dr Joynson comments:

We first brought attention to the problem of false positive results occurring with heat-inactivated sera in an *indirect* ELISA for toxoplasma Specific IgG in 1987[1] and suggested that it could occur with indirect ELISA's for other agents. The work of Gombošová and Valent substantiates this. We would, however, point out that the problem seems to be confined to *indirect* ELISA methods—that is, where antigen is coated on to the solid phase. In our experience false positive results do not occur with heat inactivated sera when tested for toxoplasma specific IgM by an antibody class capture type assay.

Reference

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Manual and automated prothrombin time tests

I was interested in the paper by Poller *et al* and a little surprised by their finding that manual prothrombin time tests show greater precision than automated tests in the National External Quality Assessment Scheme (NEQAS) survey.[1] Intuitively, I would have expected the opposite. Their findings seem to contrast greatly with the situation in North America.

Unfortunately, the international normalised ratio has not yet been widely adopted here and the international sensitivity index of reagents commonly used is quite different, so that a direct comparison cannot be made. On the basis of the College of American Pathologists (CAP) survey,[2] however, in which a large number of laboratories in the USA and Canada participate, there seems to be a much higher degree of precision in automated prothrombin time results compared with that shown by the NEQAS results.

In the CAP survey, participants are grouped according to the reagent/analyser combination used. Review of the results for 1988 shows the most common combination to the Dade Thromboplastin C with the MLA Electra 700. This was used by more than 250 laboratories to analyse 15 survey samples with prothrombin times in the range 10–24 seconds. In no case did the coefficient of variation (CV) exceed 4%. The Coagamate X-2, in combination with General Diagnostics Automated Simplastin, was used by over 30 laboratories and the CV for all samples tested was less than 5%. Review of all reagent/analyser combinations used by 20 or more laboratories showed that most had a CV of less than 5%.

Analysis of manual prothrombin time data is not available as manual methodology is almost extinct as a means of routine coagulation testing, and as already mentioned, direct comparison with the NEQAS results may not be appropriate. None the less these results suggest that there may be greater precision with automated methods as used in North America. No doubt, there are a number of possible explanations for this. One which occurs to me is that there may be interbatch variation in the thromboplastin used by NEQAS participants, at least in relation to its performance in an automated system.

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1 Poller L, Thompson JM, Taberner DA. Effect of automation on prothrombin time test in NEQAS surveys. *J Clin Pathol* 1989;**42**: 97–100.
2 College of American Pathologists' Surveys. *Comprehensive Coagulation Module Survey* Washington, DC: CAP, 1988.

Dr Poller comments:

Dr Easton makes two relevant comments regarding North American and United Kingdom quality control results in prothrombin time testing. The first is his surprise at the better precision of manual *versus* automated results in the United Kingdom surveys. The long established proficiency in the manual technique in the United Kingdom is well recognised and may relate to the centralisation of coagulation procedures in the United Kingdom in district general hospitals and other large laboratories.

The second point concerns the relative precision (CV) in prothrombin time testing obtained in recent United Kingdom and College of American Pathologists' (CAP) surveys. The CV is influenced by the difference in sensitivity of reagents used in the United Kingdom compared with those in North America. The former, with ISI values close to 1·0, give considerably longer prothrombin times on Coumarin treatment and hence higher CV. For meaningful comparison of precision, however, the results must be expressed on a uniform scale using INR. We have shown that the CV of the INR approximates to the CV of the PT ratio × ISI. For example, with a thromboplastin with an ISI of 2·0 (most North American reagents have even higher ISI values), a CV of 4% for a prothrombin time expressed in seconds, quoted for the North American surveys by Dr Easton, increases to 8% when expressed as INR. In contrast, as most hospitals in the United Kingdom use a thromboplastin with an ISI of 1·1 the effect is relatively insignificant. Thus when results are reported as INR precision with this thromboplastin is at least as good as with American reagents.

Responsive thromboplastins with an ISI close to 1·0 have been shown to give greater safety in anticoagulant dosage.[3] They also provide at least equal precision in testing to high ISI reagents using the INR system, irrespective of whether a manual or automated technique is used.

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2 Turpie ACC, Hirsch J, Gunstensen J, *et al.* Randomised comparison of two intensities of oral anticoagulant therapy after tissue heart valve replacement. *Lancet* 1988;i:1242–5.

Morphometric analysis of suprabasal cells in oral white lesions

Shabana *et al* compare what they describe as *two* morphometric indices of cell shape.[1] They define the "first" of these the form factor (PE) given by:

$$PE = 4 \times \frac{22}{7} \times \frac{\text{cell area}}{\text{cell perimeter}^2}$$

The "second" of these, the contour index (CI), is given by:

$$CI = \frac{\text{cell perimeter}}{\sqrt{\text{cell area}}}$$

Both of these are indices of departure of any outline from a perfect circle[2] that are in widespread use to describe shape.[3] Although superficially, the two formulae seem to be different—PE for a circle being 1 while CI for the same shape is 3·54 ($=2/\sqrt{\pi}$)—it is clear that both indices are a ratio of perimeter[2] to area with some constants included in the former case.

Indeed, if we substitute the second formula into the first we get:

$$PE = (4 \times \frac{22}{7})/CI^2$$

These indices are therefore not separate but a fixed mathematical function of each other, both describing the same features of shape in the same way. It is not surprising that the authors state, "the contour index seemed to have a similar pattern to the factor PE."

In defining the indices separately and devoting a section of the discussion to comparison between them, the authors create a misleading impression that they are measuring different attributes. A range of morphometric shape descriptors has been advocated as having genuine differences in properties,[4] some of which are better detectors of departure from circularity than the relatively insensitive[2] form factor.

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2 Young IT, Walker JE, Bowie JE. An analysis technique for biological shape. I. *Inform Control* 1974;**25**:357–70.

3 Crocker J. Morphometric and related quantitative techniques in the study of lymphoid neoplasms. A review. *J Clin Pathol* 1984;**143**:69–80.

4 Gschwind R, Umbricht CB, Torhorst J, Obergolzer M. Evaluation of shape descriptors for the morphometric analysis of cell nuclei. *Path Res Pract* 1986;**181**:213–22.

Dr Shabana comments:

I would like to advise that our work started in 1982 when the two described factors were available. Whereas form PE was a "built-in" parameter in program Videoplan of the IBAS-1, CI was described in the literature. The practical experiment using the two factors showed differences in reproducibility, PE being more reproducible than CI. This could be explained by the differences in reproducibility in measuring the perimeter which would change the results of these factors to a greater extent than similar changes in area. The results then reflect the accuracy of the system, including the operator.

ISI value of thromboplastin

Taberner *et al* argued that the ISI value of a thromboplastin strongly influences the interlaboratory variability of the INR obtained with it.[1] A few comments on this are appropriate. The authors derived the equation CV(INR) = CV(PR) * ISI in which CV(PR) is the interlaboratory variation of the measured prothrombin time ratio. In this equation the variation of the ISI and the biological variation among individual anticoagulated patients are not accounted for. The purpose of external quality assessment is to determine the deviation of an individual laboratory's test result from the true value.

Multicentre calibration studies have shown that there is variability of the ISI.[2] If the variation of the ISI is also taken into account, the total variation of the INR may be obtained as:

$$CV(INR) = \sqrt{(CV(PR) \bullet ISI)^2 + (\ln(INR) \bullet CV(ISI))^2}$$

It should be noted that if the total variation of the INR of an individual patient's sample is to be assessed, the biological variation of the INR among patients should be included as well.[2]

My second comment relates to the observed difference of CV(INR) between two different groups of laboratories using different (rabbit) thromboplastin reagents. A CV of 13% was obtained for the laboratories using a reagent with an ISI of 1·4, and a CV of 9% was obtained for the other group using a reagent with an ISI of 1·1. Although