Comparing substrates for the detection of ANAs

The article by Pollock and Toh1 about the detection of antinuclear antibodies (ANAs) using Hep-2 cells transfected with Ro/SS-A raises the important question of the optimal method for screening of sera from patients with suspected autoimmune connective tissue disease. We have directly compared the performance of the same Hep-2 transfected cells (Hep2000; Immuno Concepts, California, USA) with Hep-2 untransfected cells (Quantflour; Sanofi Diagnostics Pasteur Inc, Minnesota, USA). The results from our study combined with a reassessment of Pollock and Toh’s data cast doubt on their conclusion that Hep-2 transfected cells are more reliable than other substrates for detecting clinically meaningful ANAs.

Sera from 258 patients referred to our laboratory for ANA testing were analysed for the presence, titre, and pattern of ANAs using Ro/SS-A transfected and untransfected Hep-2 cells. Indirect immunofluorescence was performed at a screening dilution of 1/40 assayed on 1/2560, and slides were reviewed independently by at least two scientists. In general, the correlation between the two substrates for detection of ANAs was good (table 1); discrepancies that are unlikely to be of clinical relevance were found for low titre positives. The only significant difference between Hep-2 transfected and Hep-2 untransfected cells was that the latter were more sensitive for ANAs at high titres.

Pollock and Toh report that seven of 110 Ro/SS-A positive sera did not have a positive ANA pattern in the background non-hyperexpressing cells; in contrast, we found speckled ANA staining using the untransfected Hep-2 cells (one in 11 anti-Ro/SS-A positive samples). One patient with alcoholic liver disease was repeatedly negative on Hep-2 untransfected cells but Ro/SS-A positive on Hep-2 transfected cells. This patient’s serum was analysed for antibodies to extractable nuclear antigen (ENA). Enzyme linked immunosorbent assay (ELISA) (ENA RELISA; Immuno Concepts) detected anti-SS-A antibodies at a concentration of 33 ENA units. However, the patient does not have any features of an autoimmune connective tissue disease and testing of his serum by counterimmunoelectrophoresis and line immunooassay (INNO-LIA ANA; Innogenetics NV, Ghent, Belgium) confirmed the absence of anti-SS-A antibodies.

Pollock and Toh also conclude that the positive predictive value and specificity of detection of anti-Ro/SS-A antibodies on Hep-2 transfected cells is 100% and 91%, respectively, by comparing detection by immunofluorescence with detection by ELISA. This conclusion is questionable, because the specificity and predictive value of the test should depend on a correlation with the clinical diagnosis rather than another diagnostic test. Although it is useful to know the concordance between Ro/SS-A detected by Hep-2 transfected cells and ELISA, the true clinical usefulness of the test lies in its specificity and positive predictive value for the associated clinical conditions. In fact, the true specificity of the test can be calculated from the data provided in table 3 of their paper, and is only 77% (64 of 83 patients positive for Ro/SS-A) for the diagnosis of systemic lupus erythematosus and Sjögren’s syndrome, with a sensitivity of 89% (64 of 72 patients).

Together with our data, these figures indicate that the Hep-2000 substrate in fact performs suboptimally, and like ELISAs that detect anti-Ro/SS-A antibodies in up to 17% of normal sera, appears too sensitive for reliable diagnosis in autoimmune connective tissue disease. Furthermore, the advantage of using the transfected cell line for initial screening is likewise questionable, because not only do anti-Ro/SS-A positive sera detected by immunofluorescence need to be checked for additional ENAs by other methods, but 15 sera testing positive for anti-Ro/SS-A antibodies by immunodiffusion were negative by immunofluorescence in Pollock and Toh’s study.

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The authors reply

First, we would like to point out that the cohort of sera screened for antinuclear antibodies (ANAs) by Williams et al (258) is considerably smaller than that screened by us (10500). 2 The incidence of ANA negative Ro detected on conventional Hep-2 cells (one in 11) is comparable with our report of seven in 110 ANA negative Ro. Third, although the one ANA negative Ro

reported by Williams et al did not appear to have autoimmune connective tissue disease, it cannot be implied from this very small study that all ANA negative Ro do not have this disease. Fourth, we have made no claims to the specificity of the assay as stated in the commentary by Williams et al. We were at pains to avoid the use of this term. Clearly, Williams et al have misread our text. We have commented on the sensitivity of the assay and not its specificity. They also appear to have misread our findings with respect to the 15 sera that tested positive by immunodiffusion, which they refer to in their letter. Ro was missed in 14 of these 15 sera not because the test was not sensitive for immunofluorescence but because they gave strong immunofluorescent staining and high titre ANAs, which we suggested might have masked the presence of Ro. Fifth, table 1 as presented in their letter is not informative because it does not give the number of tests that are Ro positive using the Hep2000 substrate. Finally, the conclusions of Williams et al that the Hep2000 assay performs suboptimally and is too sensitive for reliable diagnosis of autoimmunity in cases of autoimmune disease is unfounded. The specificity of the assay of 77% as calculated for us by Williams et al based on our data is respectable. Furthermore, there are currently no published data (and in 1999 Williams et al certainly have not provided any data, other than the single ANA negative Ro) that the test is too sensitive for routine diagnosis. In closing, we stand by our conclusion that Hep2000 provides a suitable substrate for the routine and ready detection of Ro positive sera by immunofluorescence.


Table 1  Results of indirect immunofluorescence at dilutions of 1/40 and 1/160 using Hep2 and Hep2000 as substrates for sera from 258 patients*

<table>
<thead>
<tr>
<th></th>
<th>1/40 Hep 2</th>
<th>Hep2000</th>
<th>1/160 Hep 2</th>
<th>Hep2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>120</td>
<td>130</td>
<td>Negative</td>
<td>158**</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>78</td>
<td>Specified***</td>
<td>44</td>
</tr>
<tr>
<td>Homogenenous</td>
<td>55</td>
<td>33</td>
<td>Homogenenous</td>
<td>38</td>
</tr>
<tr>
<td>Nucleolar</td>
<td>12</td>
<td>12</td>
<td>Nucleolar</td>
<td>11</td>
</tr>
<tr>
<td>Centromere</td>
<td>5</td>
<td>6</td>
<td>Centromere</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>9</td>
<td>Other</td>
<td>9</td>
</tr>
</tbody>
</table>

*Sera from some patients had more than one ANA pattern.
**12% difference (95% confidence interval, 2% to 22%; p = 0.03).
***Includes Ro/SS-A pattern on Hep2000.

ANA, antinuclear antibody.
flation, or the consequences of hypovolaemia. Blood may be taken in the first two to three days after death to confirm raised mast cell tryptase, which is released during anaphylactic or anaphylactoid reactions. Caution is required in interpreting mast cell tryptase concentrations because they can be increased after exposure to opiates. IgE against specific allergens can be sought in postmortem blood samples. The absence of specific IgE cannot rule out allergy as the cause of death, but confirmation of sensitivity to specific agents may add weight to a diagnosis of anaphylaxis. Unfortunately, specific IgE testing is not available to many anaesthetic or antibiotic drugs. We advise collecting samples of clotted and EDTA anticoagulated blood as soon as possible after death and guidance from an expert laboratory.

Is postmortem testing for anaphylaxis important? We believe that collecting important data on drugs and other interventions requires such examinations to be carried out. For example, collection of data on a series of mishaps related to desensitisation led to improved guidelines for this procedure and a reduction in mortality. We are unaware of data on drugs and other interventions required in interpreting mast cell tryptase concentration.

The absence of specific IgE cannot rule out allergy as the cause of death, but again in this situation, assiduous collection of data may form an important part of a legal defence.

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2 Burrow GH, Vincent KA, Krabich J, et al. Latex allergy in non-spina bifida patients: unfa-
3 Edston E, van Hage-Hamsten M. Beta-tryptase measurements post-mortem in anaphylactic deaths and in controls. Forensic Sci Int 1998;93:
135–42.

The authors reply

We thank Drs Helbert and Robinson for pointing out this area that we did not cover in our review,1 and the useful information contained in their letter. It is obviously impossible to cover every aspect of the investi-
gation of deaths following anaesthesia but anaphylaxis, although rare, is important and we will include it in future versions of the ACP Best Practice Guidelines.

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1 Start DR, Cross SS. Pathological investigation of death following surgery, anaesthesia, and medi-

### Notices

**British Society for Clinical Cytology**

**39th Annual Scientific Meeting, Leicester, UK**

10–12 September 2000

The meeting will consist of symposia, workshops, and lectures with preferred papers and posters. A full social pro-
grame will include the Society Dinner on the Monday evening. Costs: £130 for the whole meeting with £70 daily regist-
ration.

Further details: Scientific Meetings Of-
icer, RCPath, 2 Carlton House Terrace,
London SW1Y 5AF; tel +44 020
7451 6739/6740;
email: leslie.couch@psilink.co.uk

**Muscle Disorders: Pathophysiology**

**Applications and Techniques in Veterinary Pathology**

27 September 2000 and 5 October 2000, respectively

Two one day symposia held at the Royal
College of Pathologists, 2 Carlton House
Terrace, London SW1Y 5AF.

The above meetings are open to members
and non-members of the College.

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London SW1Y 5AF; tel +44 020
7451 6739/6740;
email: leslie.couch@psilink.co.uk

**FNA Cytology Using the Cytospin Method**

11 October 2000

Chorley Hospital, Preston, UK

This biennial one day course is aimed at consultants and trainees in histo-
cytopathology, and cytology BMS. The
morning session will include lectures,
with practical aspects in the afternoon.
A large teaching collection will be available for viewing, with tuition by experts in the field. The course will take place at the
Chorley Hospital part of the Preston
Pathology laboratory. Costs: £60 to in-
clude lunch, tea, and coffee.

Further details: Dr Aj Howat, Depart-
ment of Histopathology, Royal Preston
Hospital, Preston PR2 4HG, UK; tel +44
01772 710141; fax +44 01771 710181;
email: Alec.Howat@btinternet.com

### International Society for Diagnostic
Quantitative Pathology

**XIIIth International Congress**

6–20 October 2000

Hilton International Hotel,
Adelaide, Australia

Quantitative diagnostic pathology is a field of applied science addressing the problems of diagnosis and prognosis of disease by producing robust objective solutions. The conference theme “Quan-
titative diagnostic pathology in the infor-
mation age” reflects the maturity of the discipline and the need to standardise the various methods now used. The sessions will reflect the latest thinking and research in the area as well as introducing the use of quantitative methods, data processing techniques, and signal processing in the area of molecular pathology.

The programme of scientific presenta-
tions and poster sessions outlining new and evolving work is supported by a series of lectures on the state of the art and the importance of new directions. The keynote speakers are leading figures in the fields of quantitative pathology, molecular pathology, applied mathematics and statistics, and sensor and information signal processing, as well as clinicians providing impor-
tant problems in diagnostic pathology that need to be resolved from their perspective.

Over the course of the week there will be several scientific sessions concentrating on specific themes. These include follow-
standardisation and quality control in quantitative pathology; quantitative genetic/molecular pathology; data evalua-
tion and analysis; signal processing; data evaluation “workshops”; prostatic pathol-
ogy; implementation of quantitative pa-

thology in routine diagnostic pathology;
gynaecological and breast pathology; gastrointestinal pathology—diagnosis and predicting outcomes; angiogenesis; quanti-
titative pathology and lymphomas.

Two special symposia will also be organised focusing on “new advances in quantitative pathology” and “web based environments for supporting the learning of pathology”.

Registration fee: before 31 July, A$500 (students/registrars A$450); after 31 July and before 6 September, A$580 (students/registrars A$500).

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*J Clin Pathol* 2000 53: 565
doi: 10.1136/jcp.53.7.565

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