

Letters

Rationalised virological electron microscope specimen testing policy

I read the two letters from McCaughey and Curry and their respective colleagues^{1,2} with astonishment. Their debate on a policy for electron microscopy (EM) use reminded me of those mediaeval ones about the number of angels that could dance on the head of a pin. Interesting debate, pity it missed the point. They failed, I think, to address three important aspects:

(1) The nature of virus diagnosis. No one can pretend that virus laboratories can investigate every individual "viral" illness in the community, but they have an obligation to monitor what is prevalent in it. Although attractive to a cash strapped service, selecting specimens on the basis that some are more likely to yield positives than others misses the point of diagnosis—more than one virus can cause many "virus like" syndromes and virus excretion does not parallel exactly the presence of symptoms. To discard specimens taken from those who are recovering because the yield may be low, or where the cause is apparently obvious, strikes me as arrogant. If someone has taken the trouble to send a specimen, it seems reasonable to look at it, if only in the hope that the sender might be encouraged to send others in the future, especially if it turns out to be positive. It is a constant battle to get worthwhile virological specimens sent to the laboratory—choking them off is daft. Moreover, I would further confirm the Irish view that solid stools may often yield positives and also that making the diagnosis by holding the specimen up to the light (metaphorically speaking) is very unrewarding. Trends in infection can only be given some credence if the specimen base remains more or less constant. Arbitrary and variable selection of what will be examined, and what will not, destroys this base. Elaborating "a rational policy" is always a recipe for cutting down what is done (why else have one?), usually in pursuit of saving money, and is rarely based on sound science.

(2) The question of money. EM is unique among virological laboratory techniques in that the major costs of using it diagnostically (equipment, staff) are incurred in setting it up in the first place. Running costs, in comparison, are trivial, but savings are thought to be possible by allowing the operator to work only part time. Used full time, the technique becomes less expensive for each specimen the more it is used, but this use must be sensible, and humane to the operator. Microscopy, and EM in particular, can never really be a part time occupation—the operator needs to keep in constant practice and to be committed to it. The cream of his/her work should not be skimmed off by using other techniques (enzyme immunoassays, etc)—to detect rotavirus or adenovirus—for example. Like everyone else, electron micro-

scopists thrive on getting positive results and these reward immensely what can be a lonely working existence. Moreover, the laboratory gets several simultaneous tests for its money, results are produced more quickly than with other tests and, in most cases, with greater certainty.

(3) The need to retain EM as a non-centralised resource. With changes in travel and climate, "new" viruses can appear anywhere at any time. Part of characterising a putative new virus, whether truly novel or merely transferred to a new habitat, is to know what it looks like—other properties correlate surprisingly well with structure—and having only a national, or even a regional, facility for this purpose is usually a recipe for serious delay. Specimens from the periphery do not carry the same urgency as the facility's own work, especially if the latter has to meet internal performance targets. Passing perhaps the most interesting part to another laboratory is hardly likely to encourage the local virus hunters either.

Clamping EM into a "rational policy", instead of encouraging its use wherever possible, will ultimately cause it to die a slow death from a downward spiral of discouragement. Put in rugby terms, use it or lose it. EM is the only truly "catch all" technique in diagnostic virology. If lost, it will be replaced (partially, even at best) with a battery of tests which, collectively, will cost more, take longer to do, and will frequently miss the dual or triple gut infections that are common in children.

1 McCaughey C, O'Neill H, Wyatt D, *et al*. Rationalised virological electron microscope specimen testing policy [letter]. *J Clin Pathol* 2000;53:163.

2 Curry A, Morgan-Capner P, Caul EO. Authors' reply [letter]. *J Clin Pathol* 2000;53:163.

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The authors reply

Madeley's perception of the continuing usefulness of diagnostic electron microscopy (EM) in virus laboratories is very different from ours. We believe that diagnostic EM is becoming largely redundant and will continue to be replaced by other methods. Increasingly, these alternatives are molecular. Such assays are preferable to EM on grounds of cost, sensitivity, and the inherent versatility of generic methodology. In our laboratory, we have replaced EM as our routine first line test for adult cases of gastroenteritis with nested reverse transcription polymerase chain reaction (RT-PCR) for small round structured virus (SRSV). We have replaced EM as the first line test for skin material with a multiplex nested PCR for herpes simplex virus (HSV) types 1 and 2 and varicella zoster virus (VZV).¹ This approach has yielded a sixfold increase in sensitivity over EM.¹

Madeley appears to confuse the cost of expensive commercial molecular assays with that of much more economical in house assays. The change from the use of EM and virus isolation to nested PCR in our laboratory was on the basis that the latter approach was more economical, in addition to the improved assay performance.² A typical reagent cost for our in house nested PCR

assays would be less than £3, including extraction, and the manpower costs are considerably less than for traditional methods.² The cost of EM is not "trivial"; the man hours spent are a valuable resource that can be used more productively within the diagnostic laboratory.

Molecular assay delivery does not require operators with more training or experience than traditional methods such as EM, virus isolation, and antigen detection. The use of generic methodology simplifies the training and skill mixes required to provide a comprehensive service compared with that required for a service delivered via diverse traditional methods.

The term "catch all" was first applied to diagnostic EM in the 1970s and is now inappropriate. EM is insensitive and non-specific. Our nested multiplex PCR assay can distinguish between HSV-1, HSV-2, and VZV in a slide from a vesicular skin lesion. EM will often miss the diagnosis, and even when positive will only indicate the presence of a virus from the Herpesviridae family.

In 30 years of diagnostic EM we have not discovered any new viruses. In the first year of routinely using nested PCR on all skin specimens we have discovered a deletion mutant of HSV type 1.³ Looking for new viruses via routine diagnosis is not a function of a diagnostic virology laboratory. Producing results that affect patient management and infection control in an efficient, timely, and economic fashion is a major function.

If Professor Madeley considers the rewarding aspect of diagnostic virology to be the positive result, then he should welcome the availability of more sensitive methodology. There is a responsibility for diagnostic laboratories continuously to improve their performance, rather than to continue with assays whose appeal is aesthetic rather than functional.

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1 Jain S, Wyatt D, McCaughey C, *et al*. Nested multiplex polymerase chain reaction for the diagnosis of cutaneous herpes simplex and herpes zoster infections and a comparison with electronmicroscopy. *J Med Virol* [In press.]

2 Coyle PV, Desai A, Wyatt D, *et al*. A comparison of virus isolation, indirect immunofluorescence and nested multiplex polymerase chain reaction for the diagnosis of primary and recurrent herpes simplex type 1 and type 2 infections. *J Virol Methods* 1999;83:75-82.

3 Coyle PV, Jain S, Wyatt D, *et al*. Description of a nonlethal herpes simplex virus type 1 glycoprotein D deletion mutant affecting a site frequently used for PCR. *Clin Diagn Lab Immunol* 2000;7:322-4.

The authors reply

Professor Madeley's comments are very welcome but his letter opens up the debate about electron microscopy (EM) to a much greater extent than that originally intended by either the rationalised EM policy paper¹ or the recent correspondence relating to it.^{2,3} We strongly agree that EM should not be allowed to wither and die, but maintaining the relative abundance of EM units that existed into the late 1980s was untenable for two main reasons. First, the development of new investigative and diagnostic technologies (initially

enzyme immunoassays and subsequently the polymerase chain reaction) and secondly financial pressures that have affected, not only the PHLS, but also hospital pathology departments and universities. Both these factors have meant that the use of EM in many centres has been critically reviewed. Many universities have centralised their EM facilities into units either specialising in materials science or biomedical science. Within pathology, many laboratories have given up locally provided EM services because of cost considerations and buy only the EM services that they require from established units, which is more cost effective than maintaining a local EM facility. Within the PHLS, a strategy for EM was formulated, which resulted in the formation of a strategic network of EM units in England and Wales being retained. This rationalised EM service provided significant improvements in virus surveillance and showed—for example, the true importance of small round structured viruses (SRSVs) as a cause of outbreaks of gastroenteritis in the UK.

A fundamental limitation of EM is that every specimen needs individual examination by a skilled microscopist. This aspect of EM cannot be automated and restricts the number of specimens that can be examined by a microscopist within a working day. Only those specimens that warrant individual attention should be examined, and this is at odds with Professor Madeley's view that if a specimen is submitted it should be examined. Newer diagnostic methods are very sensitive, much cheaper for each test, can handle greater numbers, and often require less skilled staff to perform them. It is because of such developments that EM would no longer be considered as a front line test for—for example, group A rotaviruses.

Professor Madeley also makes the point that EM is relatively cheap after the initial capital investment. However, this is over simplistic because microscopes and associated ancillary equipment all have finite lives. Even if an electron microscope were in operation for 20+ years, few organisations would have the money to replace such an instrument on anything near a one to one basis. Even in the remaining microscope units, the cost of annual maintenance contracts for such instruments is high, and when taken with the limited number of specimens that can be examined and the costs of a skilled electron microscopist, this means that a virological examination costs tens of pounds to perform. We would agree that EM is a "catch all" method, but specimen numbers and turnaround times must be considered if an efficient diagnostic service is to be provided to the customer, who has to pay for such investigations.

What we have tried to achieve with our specimen testing policy is to provide a range of diagnostic techniques to our customers, which necessitates testing only the most appropriate specimens by EM; this is underpinned by epidemiological evidence of the relative benefit of discriminating between samples as opposed to the "shot-gun" effect of unconsidered and unstructured sampling.

EM does have a future, but this probably lies with units that have a broad range of ultrastructural interpretative skills, carry out several preparative methods (such as negative staining and thin sectioning), and have a high throughput of specimens, thus maximising

the use of these expensive facilities. Units able to undertake a variety of work, with skilled interpretation, will prosper. However, an important problem in the medium term is how to pass on established EM skills to a new generation of electron microscopists. Few organisations that have retained EM facilities have grappled with this problem of succession planning.

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- 1 Curry A, Bryden A, Morgan-Capner P, *et al.* A rationalised virological electron microscope specimen testing policy. *J Clin Pathol* 1999;52:471-4.
- 2 McCaughey C, O'Neill H, Wyatt D, *et al.* Rationalised virological electron microscope specimen testing policy [letter]. *J Clin Pathol* 2000;53:163.
- 3 Curry A, Morgan-Capner P, Caul EO. Authors' reply [letter]. *J Clin Pathol* 2000;53:163.

Aberrant CD10 expression by NHL

The report by Millar and colleagues¹ of aberrant expression of CD10, the common acute lymphoblastic leukaemia (ALL) antigen, by the cells of a marginal zone non-Hodgkin's lymphoma (NHL) is important because it reminds histopathologists not to rely too heavily on the results of single surface marker tests. "Aberrant" expression of CD antigens by malignant lymphohaemopoietic cells is a phenomenon with which haematologists have long been familiar; expression of "myeloid" antigens CD13 and CD33 by the blast cells of common ALL and the "B lymphoid" antigen CD19 by those of acute myeloid leukaemia with the t(8;21) translocation are two classic examples. But is not the range of CD10 positive NHL cases already rather broader than they suggest? We have known for years that up to 20% or so of lymphoblastic lymphomas have an immunophenotype indistinguishable from common ALL and that a similar proportion of T cell lymphoblastic lymphomas, albeit not acknowledged by the REAL classification,² may also express CD10. In addition, the REAL classification also points out that some cases of mantle cell and diffuse large B cell lymphoma express CD10.

The range of monoclonal antibodies that work superbly on sections of formalin fixed, paraffin wax embedded tissue is steadily expanding. The wider use of comprehensive panels of antibodies in the diagnosis of NHL, whether on sections or by flow cytometry or both, will surely reveal more "aberrant" results, and may even modify our views about what "aberrant" means.

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- 1 Millar EKA, Waldron S, Spencer A, *et al.* CD10 positive thyroid marginal zone non-Hodgkin's lymphoma. *J Clin Pathol* 1999;52:849-50.
- 2 Harris NL, Jaffe ES, Stein H, *et al.* A revised European-American classification of lymphoid neoplasms: a proposal from the international lymphoma study group. *Blood* 1994;84:1361-92.

Book reviews

The Pathologist. Anim JT. (£7.50.) Square One Publications, 1999. ISBN: 1 8999 5541 0.

In the current climate of adverse publicity and a UK manpower crisis in histopathology, the book's aim to "project the pathologist and break some of the myths surrounding his profession" is most welcome. Dr Anim appears well qualified to undertake such a task, having worked in Kuwait, Ghana, and London, and having experience in the four main pathology disciplines. Despite being a histopathologist, he succeeds in minimising the necropsy fixation of most accounts and gives a considered overview of each branch of pathology, highlighting the often overlooked educational and management aspects of the job.

The book comprises a brief history of pathology and its subspecialties, analyses various training schemes and practices worldwide, and touches on future developments. It is more than just a factual account because the author puts forward opinions on the role of pathology in the undergraduate curriculum, the need for close clinical liaison, and the frustrations experienced by pathologists trained abroad who return to work in less developed countries.

The author acknowledges that some of his observations on training might be deficient owing to the speed of recent modifications which, not surprisingly, is the case in his coverage of the MRCPPath examination. Mention of the specialist training register and the concept of continuing medical education are notable omissions.

There are several appendices pertaining to training in the UK, USA, China, and the USSR, but these would benefit from the inclusion of contact addresses/web sites for organisations such as the RCPPath, ACP, IBMS, and IAP and their overseas equivalents. Although the information in the text seems up to date, the references are disappointing, with many citations of recent trends/advances dating from the mid-eighties.

The price is extremely reasonable, which encourages one to overlook the occasional typographical errors such as: "the part 2 is less formal and may consist of a dissertation".

In my opinion, the author achieves his aim and this book will be of value to those called upon to explain the work of a pathologist to health professionals or lay people, and to those involved in giving careers advice.

JOANNA S JOHNSON

Atlas of Tumor Pathology: Tumors of the Upper Aerodigestive Tract and Ear. Mills SE, Gaffey MJ, Frierson HF. (\$95.00.) Armed Forces Institute of Pathology, 2000. ISBN 1 881 04157 3.

The upper aerodigestive tract comprises the ear, nasal cavity, paranasal sinuses, oral cavity, pharynx, and larynx. The 26th fascicle in the third series of the atlas of tumor pathology, which is published by the Armed Forces Institute of Pathology, forms a reliable guide when facing the diagnostic challenges raised by diseases in these areas. The colleagues Mills, Gaffey, and Frierson, all well known for their expertise in the area of head and neck pathology, have been successful in creating a book

that goes into the required details when discussing the diseases that are specific for the upper aerodigestive tract. The growth in knowledge and proliferation of diagnostic entities associated with the upper aerodigestive tract are reflected in the fact that the current fascicle counts 455 pages, whereas the head and neck fascicle from the previous series had 343. Another difference to the previous edition is the use of colour illustrations, which greatly enhances the quality of the book.

Of course, there are some issues on which there may be disagreement with the authors. The use of the term transitional carcinoma as a synonym for undifferentiated carcinoma is confusing because in the WHO classification this definition is used as a synonym for cylindrical cell carcinoma, the tumour originally described by Ringertz in his seminal monograph. Moreover, equating psammomatoid ossifying fibroma with juvenile ossifying fibroma is unjustified: these lesions differ in histology as well as in predilection site and age. Finally, the discussion of the bony lesions occurring in the jaw is meagre but I suppose they will be the subject of a separate fascicle, as was the case in the previous series.

I can heartily recommend this book for anyone who has diagnostic responsibilities for specimens from the upper aerodigestive tract.

PIETER SLOOTWEG

CD-ROM review

The John Hopkins Atlas of Surgical Pathology. Epstein JL, ed. (£139.00 + VAT.) Churchill Livingstone, 1999. ISBN: 0 4430 7933 1

I am a lover of well written textbooks and, I suspect, like most histopathologists a relatively late adopter of new technologies such as the use of the digital image. I therefore faced the review of this CD-ROM atlas of surgical pathology with some trepidation—the reader should take this into account. This CD-ROM installs, or uses, Quick Time, Adobe Acrobat, and Folio Bound Views to present and navigate between what is stated to be over 4000 images taken from 1500 different lesion types. The images are almost all haematoxylin and eosin stained sections. The quality of photomicrography is good—illumination is even, the staining is not obviously faded, and there are no colour casts. The colour intensity is rather strong and occasionally slightly muddy. The resolution of the images is not obviously stated, but is such that a full screen image looks good, without obvious pixellation, but when the zoom in facility is used, the pixellation becomes obvious when the magnification has been increased by 50% or more. In practice, this meant that the images were best viewed at full screen and not zoomed at all.

An animated tutorial takes the viewer through the possible ways of using the atlas. I found watching the mouse arrow move around the screen by itself and press buttons slightly tedious, and aborted this programme in favour of getting started and pressing all the buttons myself.

There appear to be two main ways of using this atlas. The first is entitled “pattern recognition”. In this, one selects the desired topo-

graphic site from a reasonably comprehensive list, presented around an image of a ghostly blue coloured human body, and then screens are presented primarily by histological pattern, with possible diagnoses as the subheadings. It immediately becomes apparent that to navigate this, one has to know the name of the histological picture being looked up—if you do not know what it is called, you do not know where to start looking. Thus—for example, if you were a trainee presented with a case of crescentic glomerulonephritis but did not know what it was called, you would have to leaf through all of the pictures of glomerular disease to find out what it was called. However, once there, information about the conditions is succinct and well edited. The juxtaposition of competing differential diagnoses makes navigating this section rewarding and attractive. The second main mode of using the atlas is the “self assessment quiz mode”. In this, one again selects the desired topographical site and then brief histories are presented, together with a collection of thumbnail sketch histological images. These can then be interrogated further by enlargement before arriving at a mental diagnosis. There does not appear to be anywhere to record one's diagnosis to the computer, and to have it marked. Instead, the diagnosis is revealed after clicking on a button. This at least avoids the frustration of computer marking of free text answers, where permissible near misses get (infuriatingly) marked wrong. I found it interesting that it was reasonably easy to predict which cases were benign and which were malignant from the number of images presented. Malignant conditions can be justified within a few images. Benign ones usually take a lot of images at different magnifications to provide evidence of lack of malignancy. There is obviously “a learning curve” with the manipulation of digital images, and I found the exercise very difficult compared with direct microscopy of slides. The main problem appeared to be the slight over emphasis on high power images, the inability to select one's own field, and the lack of resolution compared with real microscopy. On the positive side, however, was the requirement to make a commitment to a diagnosis on the basis of a given field. This is obviously something that could be used in the future for testing professional interpretation in a very standardised way.

All of this raises the question “who would derive most value from the program?”. I don't think there is a lot of new information here for existing consultants. However, I can imagine the images being useful in teaching (I should note that I attempted to cut and copy some of the images out of the program, without knowing whether I am allowed to or not, but in any event, the program would not allow me to do this). Trainees might prefer this to a paper atlas because a large number of reasonably high quality images are contained here, and the indexing is fine for someone used to navigating electronic indexes. However, they would need to be at a level where they know the names of basic histological patterns. I would therefore envisage that this would be invaluable to SpRs as an adjunct to viewing slide collections and participating in bench work.

I would recommend that departments setting up an electronic learning resource for their trainees add this to the collection of available CD-ROM programs and think that practical use would be seen at least by those trainees who enjoy operating this type of technology.

TIM STEPHENSON

Notices

Muscle Disorders: Pathophysiology

Applications and Techniques in Veterinary Pathology

New Millennium Bugs

Cytopathology Update: Making Cervical Cytopathology Work

27 September 2000, 5 October 2000, 18 October 2000, and 7 December 2000, respectively

Four one day symposia held at the Royal College of Pathologists, 2 Carlton House Terrace, London, SW1Y 5AF, UK. The symposia are open to members of the college, to trainee pathologists, and to workers in other disciplines with an interest in the subject. The programme is approved for CPD.

Further details: Scientific Meetings Office, Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y 5AF, UK; tel: +44 020 7451 6740/6739; email: www.repath.org

XIII International Congress for Quantitative Diagnostic Pathology

October 2000

Adelaide, Australia

The web site for this meeting will be available at the following address from 12th May 2000: <http://som.flinders.edu.au/fusa/AnatPath/congress/main1.htm>

Practical Adult Cardiovascular Pathology Course

6–8 November 2000

Royal Brompton Hospital, Imperial School of Medicine, and National Heart and Lung Institute

This “hands on” course approaches in detail the problems that face the diagnostic pathologist when dealing with cardiovascular pathology. Congenital heart disease will be highlighted. The approach to a cardiac necropsy and sudden death will be emphasised. Cardiac specimens will be made available for analysis and practical demonstrations as well as video demonstrations. A slide seminar is also included. The course is aimed at trainees studying for the MRCPATH and also senior pathologists who wish to update their knowledge of cardiac disease, both congenital and adult. Course fee £300.00 or £200.00 for juniors in training.

Details from: Short Course Office, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY; tel: +44(0)20 73518172; fax +44(0)20 73518246; email: shortcourse.NHLI@IC.AC.UK

**Practice Guidelines for
Non-Hodgkin's Lymphoma**

21 and 22 November 2000

The Royal College of Pathologists, 2
Carlton House Terrace, London SW1Y
SAF, UK

A two day symposium that is open to
members of the college, to trainee pa-
thologists, and to workers in other
disciplines with an interest in the subject.
The programme is approved for CPD.

Course fees: fellows/members £150,
trainees, etc £90, and non-members
£200.

Further details: Scientific Meetings
Officer, Royal College of Pathologists,
2 Carlton House Terrace, London
SW1Y 5AP, UK; tel: +44 020 7451 6740;
email: [www-rcpath-org](http://www.rcpath-org)

**World Assembly on Tobacco Counters
Health (WATCH 2000)**

4-8 December 2000

India

This meeting is sponsored by the Inter-
national Congress on Oral Cancer
(ICCOOC), the International Association
for the Study of Lung Cancer (IAFTH-
SOLC), the World Heart Federation
(WHF), the Union Internationale Contre
le Cancer (UICC), and the International
Network of Women against Tobacco.

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.in](mailto:rralhan@medinst.ernet.in); website: <http://www.watch-2000.org>

Correction

**Western blotting is useful in the salivary
diagnosis of *Helicobacter pylori* infec-
tion.** Ballam LD, Mendall MA, Asante, *et al.*
J Clin Pathol 2000;53:314-17.

The authors would like to apologise for an
error that occurred in this paper. It was stated
throughout the paper that 665 children were
tested but in fact the number was 669. In
addition, in the first sentence of the results
section of the abstract 530 of 691 (87%)
should be 530 of 619 (86%). The authors
stress that these errors do not change the
message of the paper.