Letters

Rationalised virological electron microscope specimen testing policy

Curry *et al* have produced guidance on the provision of electron microscopy testing in virus laboratories.[1] Such local guidance is necessary to make expensive and time consuming diagnostic services such as electron microscopy cost-effective and clinically useful.

In their report they state that "specimens for electron microscopy examination should be unformed faecal specimens (*not* formed stools...)". They justify this approach by reference to "...20 years of experience have shown (unpublished results) that these are more productive." Effectively they reiterate the commonly accepted assumption that viruses of gastroenteritis are more likely to be found in liquid than in solid faecal specimens.

Our findings do not support this dogma.[2] We examined 2568 specimens by electron microscopy. A virus was demonstrated in 8.6% of liquid, 19.9% of semisolid, and 25.2% of solid specimens (χ^2 for linear trend, $p < 0.0001$) This observation was valid for both adenovirus (2.4%, 5.0%, and 6.6%) and rotavirus (5.2%, 13.6%, and 16.6%). Curry *et al* cite our study but consider that their impression (unpublished results) of 20 years experience is better evidence than our data. This is contrary to an evidence based approach. Curry and his colleagues should present their findings comparing formed and unformed samples from patients with gastroenteritis for peer review to substantiate their conclusions.

Before our study our experience had not led us to question that the liquid specimens were associated with the highest yield. The rationale for initiating our study was to validate rather than disprove our approach of excluding solid specimens during times of pressure of use of the electron microscope.

Our findings clearly show that solid faecal specimens at the end of an episode of diarrhoea had a higher diagnostic yield than liquid specimens taken at the peak of symptoms. This pattern of results fits closely with the biphasic pattern of excretion described for rotaviruses in various animal models.[3–5] This finding has important implications for those such as Curry *et al* and others establishing diagnostic algorithms for the investigation of viral gastroenteritis.

For diagnosis of small round structured viruses (SRSVs), it has become increasingly obvious that EM is not an adequately sensitive technique. Curry *et al* state that EM remains the front line test where SRSV is suspected. We would strongly disagree and consider nested reverse transcriptase polymerase chain reaction to be more appropriate. In our hands the sensitivity of nested PCR is 80% in specimens from SRSV outbreaks and this is now our front line test for all adult cases of gastroenteritis.

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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.
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Authors' reply

We welcome the comments of McCaughey *et al*, and agree that "experience" is no substitute for quantified scientific results that have been peer reviewed. However, the methodology used (grid floatation on a clarified 10% faecal suspension[1]) in their publication[2] is not recommended within the PHLS as a suitable procedure for concentrating small round viruses, including SRSVs, from stool samples (although the method may be adequate for rotaviruses and adenoviruses). In their study only 18 of 2568 faecal specimens were positive for SRSVs. This detection rate of 0.7% must be compared to the SRSV detection rate by EM at Manchester PHL of around 10% when using either ultracentrifugation[3] or the ammonium sulphate precipitation method.[4] It is also worth pointing out that the laboratory from which the grid floatation method originated[1] has recently sought a different method because of the relatively poor detection of SRSVs in faecal samples (personal communication to AC). The lack of sensitivity of the floatation method and the low magnification used by McCaughey *et al* to examine the grids (34 000) may explain the paucity of small round viruses detected in their study.

Despite our significant reservations about their methodology, McCaughey *et al*[2] make the point that formed stool specimens contain more virus particles than either semiformed or liquid stool specimens. The important point we made is that the specimens should be collected in the acute stage of the illness for maximum EM sensitivity when using appropriate methodology. In addition, when investigating outbreaks, we are faced with limiting the workload and this requires selection of specimens most likely to contain virus. The reality of the situation is that the laboratory usually receives only a small amount of faecal sample from suspected cases of gastroenteritis, often without adequate clinical information. Our policy would be to discard those samples that are fully formed (hard pellet appearance). At least by selecting such unformed stool specimens we know that those individuals had symptoms. In practice, if the only specimens received have been produced more than 48 hours after onset of symptoms, then we now send a selection for PCR investigation.

There is no doubt that molecular diagnostic methods for SRSV detection are more sensitive than EM and also have a significant advantage for detection of virus RNA in specimens taken more than 48 hours after the onset of symptoms. In an ideal world, with unlimited resources and availability of skilled staffing, all specimens from outbreaks thought to involve SRSVs would be tested by appropriate molecular methods and results would be available within, at most, 24 hours. For sound economic and technical reasons, the PHLS funded and developed PCR detection of SRSVs in two centres (CPHL, Colindale, and Bristol PHL, in collaboration with Southampton University). This was to ensure that the limited resources available were placed in the hands of experienced molecular biologists so that development could occur quickly and that any problems encountered were rapidly resolved. This worked extremely well and both centres now offer a PCR based diagnostic service for SRSV detection. However, results are not as immediate as electron microscopy. The PHLS EM network, with all its limitations, will remain an important part of outbreak investigation in Wales and England for the immediate future.

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Book reviews

Cerebral Ischemia: Molecular and Cellular Pathophysiology. Edited by W Walz. ($125.00.) Humana Press, 1999. ISBN 0 896 03540 9.

The mechanisms of neuronal damage and the accompanying cellular reactions that are triggered by cerebral ischaemia are reviewed. The contributors are largely based in North America, and many have a distinguished record in this field. The first contribution is a useful overview of the mechanisms of cerebral ischaemic damage, which is followed by five sections that focus on neuronal damage with particularly interesting sections on calcium overload and neuroprotection as potentially mediated by cytokines. The remaining four contributions deal with the cellular changes following ischaemia, the highlights of which for me were an illuminating discussion on necrosis versus apoptosis in neurones, and the reprogramming of gene expression in neurones after ischaemia. The text throughout the book is accompanied by black and white line diagrams and tables, with only occasional monochrome illustrations. The book is well referenced (up to 1998) and there is a helpful index.

Cerebral ischaemia is a major cause of morbidity and mortality in Western countries and so this book will be welcomed by both

clinical and basic neuroscientists, neuropathologists, and neurophysiologists. It is rather specialised for most general departmental collections, but I would recommend it as a library purchase. Over the past few weeks the book has been used by undergraduate and postgraduate students, postdoctoral worker, and neuropathologists and we have all found it helpful (without being overwhelming) and clearly presented (without being simplistic).

J W IRONSIDE

The Breast. Edited by C W Elston and I O Ellis. (£125.00.) Harcourt Brace & Co, 1999. ISBN 044303723x.

In the previous edition of *Systemic Pathology*, the breast was covered rather meagrely in a joint volume with some other tracts. In this new series, the breast has now received its well deserved separate volume. The authors, largely from the Nottingham group, have done a good job. In 24 chapters (totalling 552 pages) almost the entire spectrum of breast disease is covered. There are many pictures, all black and white almost all of high quality.

Going over all the different chapters would lead to unnecessary repetition, as all are of high quality. Let me therefore just pick out a few things that struck me and try to come up with some criticism. I particularly liked the chapters on preinvasive breast lesions, which are comprehensive and clear cut with regard to diagnostic criteria. Of course, some typical controversies remain. I would, for example, diagnose the lobular neoplasia in fig 7.8 as "atypical lobular hyperplasia" rather than "lobular carcinoma in situ". These part are quite complete, and the DCIS chapter nicely reviews the different classification systems of DCIS. I just missed a statement that papillary carcinoma may be largely solid. These chapters, however, stand a little apart, and it would have been conceptually nice to have included somewhere an overall scheme for progression of preinvasive lesions to invasive cancers, including a discussion of the putative genetic alterations involved.

The chapter on invasive epithelial cancers is also comprehensive and complete. The only remark I have here is that lymphoepithelioma, like carcinoma, could have been mentioned in the differential diagnosis of medullary cancer, and some words could have been given to the controversial role of Epstein-Barr virus in these lesions.

There is a big chapter on "The role of pathology in breast cancer", mainly addressing prognostic factors including no less than 347 references. Being no doubt biased myself, I missed some important data here and therefore found it still not comprehensive enough and a little biased towards the Nottingham results. However, I suppose the authors can be forgiven for this.

Inheritable breast cancer is briefly dealt with in the chapter "Clinical aspects of malignant breast lesions", but this deserves to have been addressed more extensively in a separate chapter. No doubt future editions will deal with these points and include recent developments in molecular analysis and important new issues such as the Sentinel Node procedure.

Despite these small criticisms, this is a very useful volume for any surgical pathologists dealing with breast lesions and can be recommended without reservation.

P VAN DIEST

CD-ROM review

GLOBOCAN 1: Cancer Incidence and Mortality Worldwide. By J Ferlay, D M Parkin, P Pisani. ($90.00.) International Agency for Research on Cancer, 1998.

Many of us are familiar with the situation where one needs some epidemiological data on a certain cancer rapidly to finish an introduction for a report or presentation. One ends up copying tables from large complicated epidemiological handbooks and wishing that there were a more sophisticated approach. This is exactly where a program like GLOBOCAN comes in handy.

According to the IARC, "GLOBOCAN is a Windows based software which provides access to a worldwide database of cancer incidence and mortality rates. It has basic graphical capabilities and provides facilities to manipulate these data." It is meant for "anyone interested in cancer epidemiology and cancer control." "The information stored is a unique resource, comprising rates of cancer worldwide estimated using methodologies developed in the unit of Descriptive Epidemiology of IARC. In addition, GLOBOCAN permits the estimation of future cancer burden, using time trends and population estimates for any country or area in the world."

Installation under Windows 98 is easy and an icon is placed in a subdirectory of the IARC directory. After installation, it is not directly clear how to proceed, but fortunately the help menu does contain a "getting started" section. This turns out to rather brief, there is no manual on the CD, and no examples are given. When we follow the suggestion to begin exploring the GLOBOCAN database using the View option on the menu, we get a menu which allows us to select a geographical region, either incidences or rates, and the option to either select or not select male or female sex. The result is two separate tables for male and female cancer incidences for 23 major sites of cancer, stratified by age. This looks nice so far, but, for example, it is not clear from the table what year is involved. Neither is it clear how to build a table with data for both sexes combined. It appears not to be possible to export tables to a word processor or spreadsheet. At first instance the graph option looks better. The program makes pie or bar charts that cannot be edited afterwards. The possibilities of the right mouse button have not yet been discovered.

Geographical maps with cancer incidences for males or females are presented per country in colour codes. However, apart from the fact that again a combined incidence for males and females cannot be displayed, the graphical presentation is also poor. Country borders are not displayed and a white colour code is even used against a white background.

The graphs cannot be exported to another program other than by copying the active window to the clipboard (Alt+PrtScr).

There are a several additional features such as population pyramids and projections of future cancer incidence rates. Also an option to create reports is available, but once a report is produced in a window, it is not clear how to save the results or export them to a word processor. There is no menu in the Result window. Clicking the right mouse button does yield a pop up menu that has an option "save as," but after selecting this option, nothing happens.

The conclusion is ambivalent; the initiative is good, and if the standard graphs or tables fulfil your needs it can be a useful program. However, the moment when one wants to do something extra, the limitations of the program are rather obvious. GLOBOCAN could have been exactly what you need, but a great deal of trial and error is needed to work with the program, and in the end you still may not have what you want. This may cause the program to end up unused on the shelf after a couple of frustrating hours.

G A MEIJER

Notices

Cytology for Pathologists

7–8 February 2000

Northwick Park Hospital, Harrow

This is an intensive course in basic cytopathology suitable for candidates preparing for the MRCPath examination and for consultants. It is conducted by the Department of Cellular Pathology, Northwick Park Hospital. The programme consists of lectures, microscopy sessions, and discussions. Topics covered include cytopathology of the cervix, urine, respiratory tract, serous effusions, and fine needle aspiration cytology of the breast and other sites. The Royal College of Pathologists has approved the course for a total of 30 CPD credits. Course fee £400.

Further details: Mrs Debbie Booth, Postgraduate Courses Coordinator, Room 6V017, Medical Education, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ; tel 0208 869 2254.

Practical Adult Cardiovascular Pathology Course

Royal Brompton Hospital, Imperial School of Medicine

6–7 March 2000

A "hands on" course approaching in detail the problems facing the diagnostic pathologist when dealing with cardiovascular pathology. Approaches to cardiac necropsy and sudden death will be emphasised. The course is aimed at trainees studying for the MRCPath.

Further details: Short Course Officer, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, UK; tel +44 (0)2073518172; fax +44 (0)2073518246; email: shortcourse.NHLI@IC.ac.uk

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2 Washington JA. Conventional approaches to blood culture. In: Washington JA, ed. *The detection of septicemia*. West Palm Beach, Florida: CRP Press, 1978:41–87.

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