for routine use. Our nested PCR gives up to 100 times the sensitivity of the single step PCR, the same amplification achieved by Southern blot hybridisation in the paper by Wakefield et al.  

We have examined samples from patients with HIV/AIDS and from other immunocompromised patient groups. Although the number of clinical samples tested was small, the results are encouraging. Of 10 HIV respiratory samples, five were positive and five negative in immunofluorescence assay. Identical results were achieved with PCR. However, in seven samples from four immunocompromised patients (non-HIV/ AIDS group) very few cysts detectable on immunofluorescence assay, PCR was clearly positive. These patients responded to specific antipneumocystis therapy. Immunocompromised patients without HIV/AIDS may have lower numbers of the organism in their respiratory secretions, in which case a sensitive assay has advantages. Our aim was to perfect a rapid sensitive PCR for routine use on respiratory samples, such as BAL or even induced sputum samples which are easier to obtain.

For Taxonomical and epidemiological investigations of *Aeromonas* sp.  

In their recent article Carey et al. contend that the universal, commercial 16S + 23S rRNA of *Escherichia coli* is an unsuitable probe for the taxonomical identification of hybridisation groups (groups 1 to 13) of *Aeromonas* sp. For more than two years, we have been studying the taxonomy of *Aeromonas* sp and have examined more than 400 strains originating—for example, from food, water, human tissues, and fish (*A. salmonicida*). Ribotyping using universal 16S + 23S rRNA labelled with digoxigenin as the probe has produced reproducible results which are comparable with those reported by Martinetti-Lucchini and Altwegg. For successful identification of hybridisation groups in *Aeromonas* sp, the isolates should have been adequately characterised by conventional methods—procedures used to purify chromosomal DNA are important because extraction of active endonucleases using phenol/chloroform and chloroform is required (polysaccharide contamination can cause problems). The quantity of DNA used is also important. Ribopatterns occurring at molecular weights of about 0.8 to 4 kilobases can be used for the identification of a hybridisation group if DNA is restricted with *Sma I*. For this area to become visible, 4 to 5 μg of DNA is needed. Carey et al. report that they used only 1 μg of DNA and I believe that this is why these authors failed to visualise “small” bands. For a molecular weight of 4 to 23 kilobases to be visualised, just 1 μg of DNA is required. This can be used for the epidemiological comparison of related isolates within a hybridisation group, as suggested by Martinetti-Lucchini and Altwegg.

M. L. HÄNNINEN  
University of Veterinary Medicine, P.O. Box 6, FIN-00085 Helsinki, Finland


Dr Carey, Eley and Wilcox comment: We appreciate the interest shown by Dr Hänninen in our recent publication. In our article we concluded that the universal chemiluminescent probe was unsuitable for taxonomical investigations but was useful for epidemiological studies of *Aeromonas* sp isolates. We have taken note of Dr Hänninen’s recommendations and wholeheartedly agreed with the first two points raised, which we complied with. It is possible that the use of more than 1 μg of DNA for endonuclease digestion could result in stronger bands in the lower molecular weight (0.8 to 4 kilobases) region. This we were somewhat reluctant to do as at times some of the higher molecular weight bands were quite strong and became difficult to visualise accurately, whilst the low molecular weight bands were hardly visible. Nevertheless, we realise that Dr Hänninen used a digoxigenin labelled probe and this may give a better balance in intensity between the high and low molecular weight bands, permitting the use of more DNA.

Contamination of crystal violet in the Gram stain method

Crystal violet is routinely used in Gram’s method for the staining of bacteria. In our laboratory all stains used in the method are filtered with a grade 113 (30 μm retention) paper filter to remove large stain deposits after preparation. Filtered stains are then stored in Winchester bottles and these are used to refill 500 ml plastic bottles in constant use at the bench.

Recently, Gram negative bacilli were seen in smears stained by Gram’s method from a number of clinically sterile sites. In most cases clinical features were not suggestive of bacterial infection. All direct cultures were negative on routine media after incubation. The possibility of contamination in one or more stains used in the Gram method was raised.

To identify the source of contamination, a representative sample of the iodine, safranin, and crystal violet solutions were centrifuged. Deposits from each were placed onto a clean, glass slide with a sterile needle and syringe and then stained by Gram’s method using freshly prepared and filtered stains. Numerous Gram negative bacilli were present in the crystal violet deposit only. These grew after enrichment and were identified as *Pseudomonas cepacia*. Further investigation revealed that the deionised water used in the preparation of the stain was contaminated with *P. cepacia* and *Corynebacterium sp*.

There have been numerous reports of contamination of laboratory reagents; one recent report found the source of contamination to be a phenol red solution. Many different organisms have been implicated in such reports, including *P. cepacia*. Contamination of hospital distilled water systems has also been reported. Deionised water is not necessarily bacteriologically sterile and should therefore be filtered with a bacteriological grade filter before use.

In this case the Gram negative bacilli did not present a problem in the interpretation of bacterial culture results. However, their presence on microscopy could be potentially misleading, especially in samples from normally sterile sites such as cerebrospinal fluid.

It is hoped that the results of this investigation highlight the problems associated with the contamination of laboratory reagents. Reagents used for staining or culture techniques in the clinical laboratory should be sterilised and filtered as far as possible using recognised techniques. Containers used to hold laboratory reagents should also be cleaned or replaced frequently.

SC CLARKE  
M McINTYRE  
Department of Microbiology, Wexham Park Hospital, Slough, Berkshire SL2 4HL


Book reviews

*Pneumocystis carinii* is among the most common infectious pathogens in humans, and its related disease *P. carinii pneumonia* (PCP) is one of the most common opportunistic infections in immunocompromised patients, especially those with AIDS. Before the epidemic of AIDS, only a few researchers were involved in the field of this so-called “enigmatic” pathogen. Peter Walzer heads one of the leading research teams on *P. carinii*. The new edition of this book perfectly reflects the tremendous advances made during the...
past decade. More than 40 authors have contributed to the 31 chapters covering the wide field extending from the bench to the clinic.

The book is organised in six parts: basic biology, epidemiology, pathophysiology, clinical features, diagnosis, treatment, and prevention. Basic data are carefully described though persisting difficulties are duly mentioned. As usual, the editors have attempted at in vitro cultivation which remains disappointing and largely contributes to slowing down progress in basic aspects of *P. carinii*. Advances in molecular biology, as well as pathophysiology features, are thoroughly described and referenced.

Half of the book is devoted to the clinical management of PCP, and provides a very valuable state of the art. The last chapters analyse new directions of drug research, including data gathered on important families of compounds like derivate of pentamidine, folic antagonists, aminoquinolines, hydroxyquinolones, and B-glucan synthesis inhibitors.

Overall, this unique book is the most comprehensive source of data in the fast evolving field of *P. carinii* infection. It will be particularly useful and time saving for researchers. Indeed, it is the reference book on pneumocystis.

PIERRE-MARIE GIRARD


This book is based on five articles previously published in *Thorax*, with further chapters added, and the result is a wide-ranging review of many aspects of lung cancer.

The first chapter goes straight to the root of the problem by describing the links between tobacco and lung cancer. In fact, although much of the book is optimistic about the advances made in the treatment of lung cancer, the overall prognosis is still very poor and this chapter makes the important point that much more effort should be made towards prevention. This is a particularly useful and informative chapter in that it goes into details of numerical trends in lung cancer and also investigates some of the politics of the tobacco industry.

Genetic changes in lung cancer are then covered. At present, there is not a great deal of information about genetic linkage in lung cancer, which is reflected in the short nature of this chapter. Some of the text would be perhaps more appropriate in the chapter dealing with the biology of lung cancer, in particular the description of p53.

There is a very useful review on the newer endocrine aspects of lung tumours. This is an aspect of lung cancer which frequently causes confusion, particularly with the terminology which is applied to small cell carcinoma. The association between the different subtypes of typical small cell carcinoma and endocrine carcinoma is clearly described and put in a historical context.

The association between hormones and growth factors and lung cancer cannot be ignored and this chapter goes into a great deal of basic biochemical detail which would probably be more than most readers would require. It is always useful, however, to review some of the basic cellular processes involved in the biochemistry of growth factors. It would have been useful to review some of the molecular biology of cell proliferation and use of proliferation markers in the assessment of lung tumours. Although chapter on lung cancer antigens, I feel, is less successful, probably because of the early nature of the development of this subject, in particular the complex nomenclature of the antigens. Time will tell if the assessment of lung tumour antigens will be relevant in the treatment of lung cancer.

The latter half of the book has a very clinical bias and is particularly useful to non-clinicians involved in the biology of lung cancer.

In summary, this book provides a good review of lung cancer, although by its very nature only snapshots of the subject are taken. The book is a multi-author book; there is some slight discontinuity and repetition within the book; a little more liaison among the authors, particularly in the basic science chapters, would have been beneficial. I would recommend it as a succinct review of some advances seen in the very important subject of lung cancer.

EA SHEFFIELD


This is a collection of papers presented at an international symposium on “fractals in biology and medicine” held in Ascona, Switzerland, 1–4 January 1993. We are told that there were 90 contributors from the USA, United Kingdom, continental Europe, Canada, and Japan. Five participants are identified as coming from Institutes or Departments of Pathology; two of these are Departments of Cellular Pathology; two others come from Microbiology Institutes. There is a section entitled “fractals in pathology” which contains four chapters; two on bone morphometry, one on the fat content of liver determined by ultrasound scans, and one on the fractal dimension of tumour/stromal interface in oral tumours. There are also papers on complex surfaces, various branching phenomena, transport processes, and biological modelling. There is no record of any discussions, although I expect that this was the most interesting aspect of the meeting. The production is of good quality, but the use of English is poor in places.

I am never sure who such conference proceedings are published for—probably the participants—and it is difficult to start any public debate about points of disagreement. Nevertheless, there are many interesting articles in this volume, for those who are interested. There is nothing here for the routine clinical pathologist; but then there is very little in the whole field of fractal geometry that has entered pathology at that level yet.

D COTTON


Of the 31 chapters in the original edition of this volume (*The Pathology of Violent Injury* 1978), 18 have been carried over into the new edition, but eight of these have been written by different authors. Demographic data have been updated in all chapters.

The editor acknowledges in his preface that the book is not meant for the specialist, raising two questions: “What is a specialist?” and “What business has a non-specialist acting in fields which require him to refer to this book?” The book that lies between two stools—it is possible for the non-specialist to fail to realise how difficult is the interpretation of some aspects of trauma and for the specialist to be frustrated by the failure of the book to address specific problems. Despite separate sections on “child abuse” and accidental injury to children, the opportunity has been missed to give details as to how one may distinguish between intentional and accidental injury to children from the injuries sustained. It is also curious that, where the book is directed to the “clinician involved in trauma”, the chapter on “asphyxia” addresses only fatalities.

As a “non-specialist” in those fields, I cannot comment meaningfully on those chapters regarding the biochemistry, haemodynamics, microbiology, and psychopathology of trauma and violence, except to point out that there is more in the literature concerning the histological dating of thrombosis than one might infer from that chapter.

An ambivalent welcome, therefore, to this new edition: should one have access to the original, purchase of this volume need not be regarded as essential.

S LEADBETTER


This is a large book which aims at comprehensive coverage of the difficult subject of pulmonary pathology. Sixty one authors have contributed, mostly from the USA, with a few contributions from Canada and South America. Layout is conventional and includes sections on the normal lung, diagnostic techniques, and paediatric lung diseases.

The text is copiously illustrated and some 200 colour illustrations are included. Unfortunately, a significant proportion of the black and white pictures are out of focus, unevenly illuminated, or lack sufficient contrast. The colour pictures suffer in some cases from poor colour rendition—eosin appearing as an intense red. Quality quibbles aside, the illustrations are abundant and well chosen.

European pathologists must expect to differ from their American colleagues over nomenclature of pulmonary disorders. Intestinal lung disease is one example and lymphomas of the lung another. The American view also prevails in the discussion of thymomas and the widely used Müller-Hermelin classification receives no mention. Excluding terminological disputes, coverage is generally comprehensive.