Dr Veenendaal and Lichtendahl-Bernards comment:
Dr Savio raises two points. The first is an alternative method of transportation, processing, and storage of gastric biopsy cultures for *H pylori*. In our article we describe a low budget method of transporting gastric biopsy samples, without the need for refrigeration or a specialised transport medium, to a laboratory facility capable of culturing *H pylori*.

Although we are surprised and impressed by the long delay without loss of viability of the culture method described by Dr Savio, we feel that this method is more tailored to a situation in which facilities (refrigeration, culture media, a microbiology laboratory) are present in the same institution. In our experience, contamination of culture plates in an endoscopy department does occur and can be a problem (especially with yeasts) when culturing a fastidious organism like *H pylori*.

The second comment addresses the important point of how to detect or better exclude the presence of *H pylori* after treatment with drugs (omeprazole, bismuth, and several antibiotics) which influence the number and viability of the bacterium (coccobacillus). As methods for detecting *H pylori* require a certain number of viable bacteria (histology, culture, and breath tests) to detect the bacterium, false negative test results are bound to occur after treatment. This problem is probably not solved by taking more samples, and is at this moment the subject of further investigation.

In previously untreated patients we found (unpublished data) no positive culture results for *H pylori* from the gastric body when the gastric antrum was also not infected. In about 20% of our patients no inflammation of infection can be demonstrated in the gastric body region, which does not support our taking of additional body biopsy specimen for routine culture in previously untreated patients.

Clinical usefulness of detecting growth of *Mycobacterium tuberculosis* in positive Bactec phials using PCR

It has recently been shown that the polymerase chain reaction (PCR) can confirm growth of *Mycobacterium tuberculosis* in Bactec phials to five days earlier than the use of DNA probes and seven to 10 days before presumptive identification by the Nomina Anatomica Parisiensia (NAP) growth inhibition test. It has been suggested that a prospective evaluation of these methods is required. We have investigated the PCR method to see if the earlier results provided would be of help in patient management.

Bactec 12B Phials are tested each morning. Those with a growth index between 20 and 50 are then read daily until the growth index falls or reaches 50, at which time a smear is made and a culture performed. The smear and subculture plates are read the following day with updated reports being sent to the clinician when acid fast bacilli are detected. Confirmed or presumptive identity is reported as soon as colony morphology, NAP growth inhibition, or DNA probe results allow. We do not use smear morphology of positive phials to generate preliminary reports to physicians.

For the three months March 1993 to May 1993, we cultured fluid from all Bactec phials with a growth index of $\geq 20$. The aliquots were stored in centrifuge tubes at $-70^\circ$C. For PCR, the aliquot was thawed and spun at 12 000 rpm for 10 minutes. The pellet was resuspended in 100 $\mu$L of a 10% chex and 1% triton solution, sonicated for 15 minutes, and heated sequentially for 15 minutes each at $50^\circ$C and $95^\circ$C. Debris was pelleted at 12 000 rpm for two minutes and supernatant fluid was used for PCR according to previously published methods. The PCR takes several hours to perform, after which it takes 90 minutes to run the gel. If PCR results from positive trials were clinically useful, we thought it might be possible to organise workflow so that the PCR result from a positive phial would be available the afternoon the phial became positive. We therefore calculated the advantage for performing PCR as if the PCR result was available the afternoon the phial became positive.

Aliquots were stored from 247 phials: 24 contained *M tuberculosis*, 48 contained other mycobacteria, 18 contained bacilli only, and 87 were sterile. The 24 specimens containing *M tuberculosis* came from 10 patients. Fifteen of the 24 (72%) original specimens containing *M tuberculosis* were smear positive. None of the 10 patients had specimens with positive smears. Aliquots from 86 phials were subjected to PCR: all 24 containing *M tuberculosis*, none of which contained bacteria; all 48 containing other mycobacteria, seven of which contained bacteria; and 14 which did not contain mycobacteria, 10 of which contained bacteria. All phials containing *M tuberculosis* were correctly identified, with a growth index of 266-2256 (range 21-999) including 12 phials with a growth index of $<100$, three of which were smear negative. It took an average of 16 days, range 6-45 days, for the phials containing *M tuberculosis* to reach a presumptive growth index. Recovery of *M tuberculosis* could have been confirmed by PCR five days (range one to 13) before presumptive or confirmed growth of *M tuberculosis* was made by other methods. No BACTEC PCR results were observed among the other 62 phials analysed. Although multiple bands were observed on the gel from one phial containing *M chelonae*, no band was positive on specific probing.

The clinical utility of the PCR result was assessed by examining the medical records of all 10 patients infected with *M tuberculosis* to determine whether the result would have enabled earlier treatment or aided in infection control measures. Nine of the 10 patients were already receiving treatment for 17 days (range three to 50) before the Bactec phial grew. All five patients with specimens with positive smears were either receiving treatment at the time or started treatment when the smear result became positive. Four patients with smear negative specimens were already receiving treatment by the time the Bactec phial became positive. Only one patient with a smear negative, culture positive sputum specimen, who was discharged the same day the phial became positive, may have benefited from the PCR result. As it was, this patient was not on treatment initiated on receipt of the phial smear result. While PCR of positive phials would have had no clinical impact, a positive PCR result on the original specimen would have been of the five smear negative patients: they would have had treatment 10 to 13 days earlier.

PCR is beginning to be compared with contemporary culture methods in large scale studies.1 The sensitivity is high for smear positive specimens, but is considerably lower for smear negative ones—94% v 62%, respectively. Although this type of evaluation is a useful first step, we should not be misled into believing that it is necessarily going to improve dramatically clinical management. For most smear positive patients, the result may not change what is done. It would be most useful for smear negative patients if the result led to earlier treatment, but even in this situation, as shown in this study, many patients are appropriately being given treatment based on clinical and family history, physical and radiological findings. We agree with the suggestions of Cormican et al that this methodology requires prospective evaluation against contemporary diagnostic methods. Such comparisons should take into account technologist time and workflow benefit for the mycobacteriology laboratory.
patients without tuberculosis may also be substantial if the need for treatment trials is reduced.

The second issue is the potential of PCR to facilitate efficient laboratory practice. Since our description of the application of PCR to the identification of M tuberculosis in positive Bactec phials, others have developed methods with broader application. Telenti et al identified a large number of species of mycobacteria by restriction digestion analysis of a 439 base pair fragment amplified from the gene for the 65 kilodalton heat shock protein. Similarly, Vaneechoutte et al have described the identification of mycobacterial isolates by restriction digestion of the amplified 16SrRNA gene and confirmed our observation that PCR can be achieved following simple heating and boiling in distilled water.

Standard methods of speciation are not only slow but technically demanding and time consuming. They also require repeated manipulation of viable dangerous pathogens. The use of DNA probes requires a different probe for each species. For these reasons we believe that PCR based methods for speciation of cultured mycobacteria are likely to facilitate laboratory practice, although the impact on clinical practice may be modest.


The multidisciplinary nature of this book, with contributions by researchers and practitioners in the fields of virology, immunology, epidemiology, pathology and clinical medicine, provides the reader with an up to date and comprehensive guide to viral infections of the heart.

The initial chapters describe the viruses associated with cardiac disease, their epidemiology, pathogenicity, and clinical spectrum. The laboratory diagnosis, both histopathological and virological, is discussed in later chapters, as well as the treatment and prevention of virus induced heart disease.

The editor concentrates, quite rightly, on the role of enteroviruses and in particular the coxsackie B viruses in acute myocarditis. Nevertheless, space is made available for the discussion of experimental evidence and clinical observations implicating a wide range of viruses, either as the cause of heart disease, or as a contributory factor. In particular, chapters on the role of HIV in heart disease. As fewer patients succumb to opportunistic infections through the use of new antimicrobial agents and improved treatment regimens, the reported incidence of heart muscle disease associated with HIV infection has risen. Chapter 8 explores the evidence, obtained mainly via molecular biological techniques, that cytomegalovirus has a role in atherosclerosis. The final chapter, which is concerned with infections with viruses and _Toxoplasma gondii_ in heart transplant recipients, describes the incidence of infection, evidence for involvement of the heart, and treatment and prophylaxis.

This well presented book sheds light on what has been a difficult and sometimes contentious area of research. The rapid progress now being made through the use of molecular biological techniques is amply illustrated throughout. This book should be read, not only by virologists, but by all those whose research or clinical practice impinges on the aetiology, diagnosis, and treatment of cardiac disease.

JJ GRAY


This is a short, down to earth, practical guide to hospital necropsies which should be of value to pathologists, trainees, and mortuary technicians. Most sections are less than 10 pages long and they concentrate on common problems, or groups of problems, on how to tackle less common situations, such as maternal necropsies. The largest section covers the routine hospital necropsy and this is supported by good black and white photographs. It is followed by a chapter on special procedures which covers a wide range of topics such as demonstrating air emboli, examining the heart’s conduction system, necropsy assessment of osteoporosis, and macroscopical dye techniques. There are specific chapters on the examination of the nervous system, fetal and perinatal necropsies, and the maternal necropsy.

Although the authors have done well to keep this book concise, I would have liked to see some tables of normal values. These would have been particularly useful in the fetal and perinatal section.

The chapter on biological safety concentrates on common microbiological hazards and gives clear information, not only on how to minimise the risks, but also the relevant regulations covering this topic (a useful resource for anyone writing their accreditation documents).

Finally, there is a chapter on clinical audit and auditing the necropsy. This should be read by all members of hospital audit committees and by all pathologists. After reading this book, we may be able to perform technically superb necropsies, but if we cannot convey the findings to the clinicians in a clear and timely manner, necropsy skills will become a dying art.

SA DILLY


This was an unusual book to review in that there was much of interest in it. But it required a lot of effort to read: it is a curious mixture of chapters. Some have information about proliferation and nothing...
Clinical usefulness of detecting growth of Mycobacterium tuberculosis in positive Bactec phials using PCR.

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