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.*¹

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 on immunofluorescence assay. Identical results were achieved with PCR. However, in seven samples from four immunocompromised patients (non-HIV/AIDS) with none or 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,³ 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.

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Taxonomical and epidemiological investigations of *Aeromonas* sp

In their recent article¹ 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 fecal samples, 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.² For successful identification of hybridisation groups in *Aeromonas* sp, the isolates should have been adequately characterised by conventional methods³—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*¹ 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 shown by Martinetti-Lucchini and Altwegg.

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- 1 Carey PE, Eley A, Wilcox MH. Assessment of a chemiluminescent universal probe for taxonomical and epidemiological investigation of *Aeromonas* sp isolates. *J Clin Pathol* 1994;47:642-6.
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Drs 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 agree 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 used routinely 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 samples from normally sterile sites. In most cases clinical features were not suggestive of bacterial infection. All direct cultures were negative on routine media after 48 hours of 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 culture 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 aquaticum*.

There have been numerous reports of contamination of laboratory reagents; one recent report found the source of contamination to be a phenol red solution.¹ Many different organisms have been implicated in such reports, including *P. cepacia*.² Contamination of hospital distilled water systems has also been reported.³ 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.

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- 1 Shears P, Rhodes LE, Syed Q, Watson J. A pseudo-outbreak of tuberculosis. *Communicable Disease Report* 1994;1:R9-10.
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Book reviews

Pneumocystis carinii Pneumonia. 2nd edn. **Lung Biology in Health and Disease.** Vol 69. (Pp 744; \$195.) Marcel Dekker Inc. 1993. ISBN 0-8247-8854-0.

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, specially 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