LETTERS TO THE EDITOR

Rapid identification of Chlamydia psit-taci and TWAR (C. pneumoniae) in sputum samples using an amplified enzyme immunoassay

Recent publications on the importance of the TWAR agent and our own experience of Chlamydia psittaci infections have led us to conduct a prospective study of routine sputum samples from patients with respiratory infections using a commercial enzyme linked immunosorbent assay (EIA) for detecting chlamydia antigen.

One hundred and six sputa were received from 100 patients with acute or persisting chest infections and with no known underlying or predisposing condition. Sera were requested whenever possible and were obtained from 30 patients. Three sputa from patients not fulfilling the trial criteria but yielding a pure heavy growth of Staphylococcus aureus were included to confirm the non-recovery of this organism in this assay.

All sputa were cultured routinely to establish their bacteriological profile. Sputum specimens for Chlamydia detection were tested by the IDEIA Chlamydia test (Novo BioLabs Ltd) and sera from patients, where available, were tested by routine complement fixation test and by a modification of a previously described enzyme immunoassay (EIA) for Chlamydia group-specific IgM. This modification required a 1 in 400 dilution of serum (100 µl) and a first incubation step of 90 minutes at 37°C. After washing, incubation with antigen took place at 37°C for one hour and conjugate was added for a further one hour at 37°C. Remaining aspects of the test were as previously described. Specimens positive by the EIA antigen test were also tested by direct immunofluorescence for direct visualisation of organisms using a specific monoclonal antibody (SYVA Microtrak) and a generic specific lipopolysaccharide (LPS) monoclonal (Imagen, Novo BioLabs Ltd).

Preparation for antigen EIA detection required mucolysis of sputum in 2% buffered n-acetyl cysteine before washing and centrifugation. The deposit was resuspended in the transport medium provided by the manufacturers. Samples positive by EIA were centrifuged as before, resuspended in PBS, and smears were examined by direct immunofluorescence for both genus and species specific fluorescence.

Results

No clinically important pathogens were detected in 79 of the 106 sputa examined. A variety of other organisms were detected including Haemophilus influenzae (n = 7), Streptococcus pneumoniae (n = 1), S aureus (n = 6), Pseudomonas sp (n = 1), and yeasts (n = 1).

Seven sputa from five patients were positive by IDEIA Chlamydia test and also by Imagen Chlamydia direct immunofluorescence but negative by SYVA Microtrak, indicating that the organism was C psittaci or C pneumoniae. In all cases the chlamydia IgM test was positive and in four cases the complement fixation titre was raised, indicating recent chlamydial infection.

One specimen was positive by all three chlamydia tests, indicating C trachomatis infection. Although the complement fixation titre was less than 16, the specific IgM was positive and species antibody was raised.

All other sputum samples were negative by IDEIA Chlamydia test including the three specimens with heavy S aureus growth. All sera from patients other than those mentioned above had undetectable specific IgM and complement fixation titres of <32.

Laboratory findings of the seven patients with evidence of chlamydial infection are shown in the table. The only finding indicating that testing of routine bacteriological sputa by IDEIA Chlamydia test may be useful for rapid detection of chlamydial respiratory infections is that this is substantiated by the direct immunofluorescence studies on the sputa and also by serology.

Although a wide variety of bacteria were encountered in this study, there were no problems with cross reactions. Four of the seven chlamydial infections described here would not have been diagnosed by routine procedures.

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Diagnosis of toxoplasmosis using DNA probes

During the past 15 years it has become increasingly evident that patients being immunosuppressed for malignant disease or organ transplantation may develop generalised toxoplasmosis which affects the central nervous system and may result in death.1 Recent findings have shown that patients with AIDS constitute a further group prone to severe disseminated toxoplasmosis: in such patients toxoplasmosis constitutes a life-threatening opportunistic infection. The current methods for the diagnosis of toxoplasmosis have given dismayingly poor results in such patients and, therefore, there is a need for more sensitive procedures for the early diagnosis of Toxoplasma gondii in body fluids or biopsy tissues. A good alternative to the serological tests may be the detection of the pathogen’s nucleic acids in clinical specimens. Over the past few years the application of recombinant DNA procedures has led to the development of a number of DNA probes for potential use in the diagnosis of pathogens such as Neisseria gonorrhoeae, Plasmidum falci-parum, cytomegalovirus and enterotoxigenic Escherichia coli.2 Recent advances in recombinant DNA technology, and in particular the development of the polymerase chain reaction (PCR),3 allow certain pathogens to be detected which may be present in very small numbers in a given sample. We wanted to develop a DNA based test for the diagnosis of T gondii. We used the PCR (30 cycles) and two oligonucleotide probes (20-mers) based on the published sequence for the toxoplasma P30 gene to amplify toxoplasma DNA. These reactions were performed with purified toxoplasma DNA (0-100 ng) in the presence of absence of 5 µg human DNA (this corresponds to about 1×10 6 leucocytes). On electrophoresis of the reactions on 0.8% (w/v) agarose gels and subsequent staining of the gel with ethidium bromide, amplified product (915 base pairs long) could be seen in those reactions contain-
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