Chapter 13
Chlamydophila

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13.1 Summary of Methods

Genus specific and species specific sequences should be used. An algorithm was devised to detect firstly the *Chlamydia* genus and then to speciate with *Chlamydophila pneumoniae* and *Chlamydophila psittaci* specific primers.

These have generally been based on the MOMP gene (major outer membrane protein) and the 16 s RNA for genus and genes from the 16 s RNA region for species. A more recently described species specific target for detecting *C. psittaci* is the *inc*A gene. PCR based genotyping for *C. psittaci* is available.

13.2 Clinical Background

Chlamydia (*Chlamydophila*) are obligate intracellular organisms producing oculo-genital disease (*C. trachomata*) respiratory and systemic infections (*C. pneumoniae, C. psittaci*).

C. pneumonia is a primary human pathogen responsible for lower respiratory tract infection. It causes acute and sub-acute respiratory disease, e.g. Pneumonia, sinusitis, bronchitis and exacerbations of obstructive lung disease. It is associated with asthma particularly the adult onset form of the disease. It has been linked inconclusively to some chronic diseases such as acute coronary artery disease (CAD) and neurological conditions such as multiple sclerosis and Alzheimer's disease and has been detected in tissues such as unstable coronary artery plaques and brain tissue involved in chronic neurological conditions.

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C. pneumoniae is a pathogen of variably reported prevalence. Internationally evidence of infection is common. Community antibody levels are up to 50–80% and 6–20% of community acquired pneumonias may involve infection with this organism. Different yields from diagnostic tests (PCR, culture, immunofluorescence and serology) may partially explain these differences. These tests are not well standardized. Quite conflicting results have been obtained associating *C. pneumoniae* and coronary artery disease, however large trials of treatment with anti Chlamydia antibiotics have not shown a benefit in CAD. Australia lacks evidence for this organism being a widespread pathogen.

C. psittaci is primarily a pathogen of birds with human infection (psittacosis) occurring rarely, usually as a result of direct bird exposure but also inadvertent bird exposure in Australian forested settings. This sporadic infection has been linked to activities such as lawn mowing. Clinical presentation is with a range of systems and organ involvement and often relatively mild respiratory symptoms. The clinical picture is dominated by fever myalgia, headache and sometimes abdominal symptoms. Patient to patient spread is rare. Mortality can be significant in untreated patients particularly the immunosuppressed and pregnant. Extensive lung involvement and multi-system failure may necessitate intensive care support. Treatment is with tetracyclines although the quinolones also are active. Macrolide antibiotics are an alternative but work somewhat more slowly. *C. psittaci* is regarded as a potential biological warfare agent.

PCR is difficult to validate on human specimens due to the rarity of illness outside of an outbreak. Avian specimens provide good control material.

Most positive human clinical specimens have DNA just at the limit of detection. Most targets are single copy genes hence copy number reflects organism number (exception *Inc*A based PCR). Sensitivity of the test is low given low copy number but specificity is high. There can be an inverse relationship between DNA presence and serological response.

Repeat infection is possible as immunity is partial. There are significant veterinary implication for *C. psittaci* both as a zoonosis and an agent of commercial bird loss. As a sporadic and epidemic cause of lower respiratory tract infection it is an important human pathogen.

13.3 Acceptable Specimens

Respiratory material including throat swabs, nose swabs, nasopharyngeal aspirates, bronchoalveolar lavage, whole blood (EDTA), urine and CSF, occasionally environmental/bird specimens.

13.4 Unacceptable Specimens

Wound swabs, drainage fluid, faeces.

13.5 Sample Extraction

Specimens are prepared as follows:

- Swabs are soaked in 200 μl of 0.9% NaCl and vortexed periodically for several hours at room temperature before DNA extraction.
- Urine is centrifuged and the pellet resuspended in 200 μl of 0.9% NaCl before extraction.
- Buffy coat from EDTA blood specimens is treated with Biorad Instagene® (Bio-Rad Hercules, Hercules, CA, USA) in order to lyse cells and inactivate DNAse and RNAse enzymes, and then resuspended in 200 μl of 0.9% NaCl.
- DNA is extracted from the specimens using the QiaAmp DNA® Mini-kit (QIAGEN, Australia), according to the manufacturer's instructions. Prior to all PCRs, bird samples are diluted 100-fold as the target organism is often too concentrated in neat specimens.

13.6 Primers and Probe Sequences

Genus specific primers used in the reaction were targeted to the 23rRNA and produced an amplification product of 168 bp [1]. The sequence for these was: CHL23SUP: 5′-GGG GTT GTA GGG TYG AGR AIA WRR CAT C-3′ and CHL23SDN: 5′-GAG AGT GGT CTC CCC AGA TTC ARA CTA-3′.

The species-specific primers and probes for *C. psittaci* were directed to the *incA* gene (74 bp) [2] and had the following sequence: *C. psittaci* Fi-inca-Cpsi: 5′-GCC ATC ATG CTT GTT TCG TTT-3′ and *C. psittaci* Ri-inca-Cpsi: 5′-CGG CGT GCC ACT TGA GA-3′. The sequence for the *C. psittaci* dual labeled probe was: 5′-FAM-TCATTGTCATTATGGTGATTCAGGA-TAMRA-3′.

Primers for the detection of *C. pneumoniae* are as follows: CPN 90: 5′ GGT CTC AAC CCC ATC CGT GTC GG-3′ and CPN 91: 5′ TGC GGA AAG CTG TAT TTC TAC AGT T-3′ resulting in an amplification product of 197 bp [3].

13.7 PCR Amplification and Product Detection

PCR is performed on a RotorGene 3000 real time PCR machine (QIAGEN, Australia) using primer sets targeting both genus (23S rRNA gene) or *psittaci* species (Inc A gene and 16-23S rRNA interspacer region).

Samples for PCR are prepared in a class 2 laminar flow hood. The reaction mixture contains 5 μl of QIAGEN® SYBR green master mix, including hot start polymerase (QIAGEN, Australia), dNTP mix and SYBR® green, 0.1 μl of primers (3 pmol/ml) and 5 μl of extracted DNA. The cycling protocol is an initial denaturation for 15 min at 95°C followed by 50 cycles of 10 s at 95°C, 15 s at 55°C, and 20 s at 72°C. Amplicons were detected using SYBR® green and melt curve analysis and confirmed with gel electrophoresis. Amplicons are occasionally sequenced

to confirm identity. An avian specimen is used as a positive control, as described. Genotyping is performed targeting variation in the OMP gene [4].

For samples testing positive for *C. psittaci* a further PCR was performed using the *IncA* gene.

13.8 Quality Control and Validation Data

DNA concentrations were determined by spectrophotometry and copy numbers calculated by reference to a standard curve that had been constructed using serial dilutions of control DNA similar to a published method [5] was found to repeatable detect 100 fg/μl of *Chlamydia* DNA.

The positive control used was a *C. psitacci* wild type avian strain provided by Professor Timms, Queensland University of Technology, Brisbane, Queensland.

Six hundred and forty human respiratory, urine or blood samples from patients displaying symptoms suggestive of *C. psittaci* were tested from 2005 to 2006. Of these 20 patients tested positive for *C. psittaci*. One of these samples was cultured and confirmed positive by DFA.

13.9 Assay Limitations

No international controls are available for *C. psittaci* due to restrictions on transport of potential bioterrorism agents. Local avian and human strains are available.

References

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