

# SEROLOGICAL DIAGNOSTICS FOR CHLAMYDIA INFECTIONS

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## Introduction

Infections with Chlamydia bacteria are a significant health problem worldwide. Sexually transmitted *C. trachomatis* infections have become rampant in recent years, especially in adolescents and young adults, while *C. pneumoniae* is a frequent cause of respiratory illness in all age groups. Although Chlamydia infections are often mild, prolonged courses can elicit long-term complications, for example infertility (e.g.

*C. trachomatis*) and reactive arthritis (e.g. *C. trachomatis* or *C. pneumoniae*).

Diagnosis of Chlamydia diseases is challenging, and laboratory methods play an important role in identifying Chlamydia infections. Direct detection by PCR or cultivation is generally the preferred method in acute infections, but has limitations, for example in long-term infections. Serological tests for sensitive and specific detection of anti-Chlamydia antibodies are a valuable tool for confirming diagnosis and for seroprevalence studies.

## Background and life cycle

Chlamydia are among the smallest intracellular gram-negative bacteria.

They infect and destroy cells of the mucosal epithelium, including smooth muscle cells, endothelial cells, monocytes and macrophages. The bacteria are energy parasites, feeding off the high-energy compound ATP of the host cells for their metabolism. They possess a unique biphasic life cycle, which is characterised by infectious but metabolically inactive elementary bodies, and metabolically active but non-infectious reticulate bodies. Nine species of Chlamydia have so far been identified, of which three are pathogenic for humans. These are Chlamydia trachomatis, Chlamydia pneumoniae and Chlamydia psittaci. Infections with Chlamydia can be easily cured with antibiotics.

## *C. trachomatis*

Non-gonorrhoeal urethritis caused by *C. trachomatis* serotypes D to K is the most frequent sexually transmitted disease. Around 700 million people worldwide are infected with *C. trachomatis*, and approximately 50 million new infections occur annually. The bacteria live mostly in the cells of the urethra. In men they are also found in the prostate or the seminal vesicles and

in women in the cervix or oviducts (salpinx). Infections are asymptomatic in around 50% of men and 70 to 80% of women. Symptomatic cases manifest in men with urethritis, epididymitis and prostatic, and in women with urethritis, cervicitis and salpingitis/adnexitis with itching, pain and discharge. Chronic infections of the inner female organs (pelvic inflammatory disease) lead in many cases to sterility, while secondary infertility in men has also been shown. There is also a link between acute *C. trachomatis* infections during the first three months of pregnancy and early abortions, premature deliveries or stillbirths. *C. trachomatis* serotypes L1, L2 and L3 cause the rare venereal disease lymphogranuloma venereum, which occurs worldwide but is most common in tropical regions.

A urogenital infection with *C. trachomatis* is followed in 1 to 3% of cases by reactive arthritis (Reiter's disease with the triad urethritis, conjunctivitis and arthritis), whereby *C. trachomatis* occurs as a metabolically active agent in the joints. The immune response is an intra-articular production of anti-*C. trachomatis* IgG antibodies.

In tropical regions, infections with *C. trachomatis* serotypes A, B, Ba and C lead to trachoma, an eye infection of varying severity. The disease is transmitted by direct contact with infectious mucous membranes of the eyes, nose and mouth. Around 400 million people suffer from trachoma and it is the most frequent cause of blindness worldwide.

### **C. pneumoniae**

*C. pneumoniae* is a worldwide distributed exclusively human pathogen which is transmitted by aerosols. Around half of infections proceed asymptotically or may cause a mild sore throat at most. All other cases are characterised predominantly by persisting unproductive cough, headache and fever. More than 50% of adults over 20 years old have been infected with *C. pneumoniae* and exhibit antibodies against the pathogen. Seroconversion is most frequently observed at an age of between 5 and 15 years old. Serious pneumonia is a risk in susceptible individuals such as the elderly, infants, pregnant women, patients with serious medical conditions and immunocompromised individuals.

As well as reactive arthritis, other chronic illnesses suggested to be associated with *C. pneumoniae* infection are bronchial asthma, coronary heart diseases and atherosclerosis.

### **C. psittaci**

Human infections with *C. psittaci* occur worldwide, but are relatively rare. *C. psittaci* is found in large quantities in the secretions and excrement of infected birds. The inhalation of infectious dust leads to ornithosis or psittacosis (parrot fever) in humans. At particular risk are bird owners and people working in the bird trade and poultry processing industries. In addition to flu-like symptoms, a life-threatening pneumonia may develop during the course of disease. This is often accompanied by further organ manifestations, e.g., of the liver, kidneys, spleen, heart, brain, joints and eyes.

### **Diagnostics**

Acute, peripherally localised *C. trachomatis* infections are usually diagnosed using direct pathogen detection by PCR. However, in chronic, ascending infections of the upper genital tract and in reactive arthritis access is difficult and direct detection is often not possible. In these cases serological methods can help to confirm an infection. Specific IgA and IgG antibodies can be detected in the majority of cases of *C. trachomatis*-caused infertility in both women and men. They are also frequently found in women who have had premature delivery or stillbirth, mostly in connection with high IgM titers. Recognised medical centres therefore recommend *C. trachomatis* screening for both parents before a pregnancy.

In *C. pneumoniae* infections, direct pathogen detection is employed to diagnose acute cases, but may fail if the infection is older. Moreover, PCR methods are not well standardised and cultivation requires considerable expertise. Thus, the serological analysis of specific IgA, IgG and IgM antibodies play an important role in the diagnosis of primary infections and reinfections. IgM antibodies are detectable around 3 weeks after symptom onset, while IgG and IgA antibodies appear shortly afterwards. Due to the high antibody prevalence in the general population, it is necessary to demonstrate a clear titer increase or seroconversion to diagnose an acute infection. This is done using two serum samples taken at an interval of several weeks. A prolonged high titer of IgA is often regarded as an indication of a persistent infection.

Serology is the method of choice for confirming a diagnosis of *C. psittaci*, since culture is only possible in specialised laboratories and PCR can be unreliable in older

infections. The first serological investigation should be performed in the acute phase as soon as possible after the onset of symptoms, followed by a second test two weeks later to monitor the disease course and a third test during treatment with antibiotics. Treatment generally leads to a decrease in antibody production, thus confirming diagnosis.

### **MIF**

The gold standard for detecting Chlamydia antibodies is microimmunofluorescence (MIF). This method employs elementary bodies of the different Chlamydia species as antigenic substrates. Cross reactivity is minimised by inactivation of the mutual outer membrane lipopolysaccharide (LPS) antigen, allowing for species-specific antibody detection. Results are evaluated by microscopy (Figure 1), whereby positive reactions are characterised by a coarse granular fluorescence, predominantly in the cytoplasm but also between the cells. In the Anti-Chlamydia MIF BIOCHIP Mosaic, the *C. trachomatis*, *C. pneumoniae* and *C. psittaci* substrates are incubated in parallel, allowing simultaneous detection of the corresponding antibodies. A control BIOCHIP of non-infected cells allows reliable differentiation between unspecific and specific reactions. The MIF provides 100% agreement with designated results for *C. trachomatis* and *C. pneumoniae* in quality assessment schemes.

### **ELISA**

ELISAs for detection of Chlamydia antibodies offer the advantage of high-throughput, fully automated processing of large numbers of samples. The Anti-Chlamydia trachomatis ELISA provides species-specific antibody detection by using the pathogen-specific native major outer membrane protein (MOMP) as the antigen. Thus, infections with *C. trachomatis* can be reliably differentiated from infections with *C. psittaci* and *C. pneumoniae*. In quality assessment schemes the ELISA yields very good agreement with the target results (IgM 100%, IgG 98%, IgA 97%).

The Anti-Chlamydia pneumoniae ELISA is based on a cell lysate containing elementary bodies and provides genus-specific antibody detection. Cross reactions with other Chlamydia species cannot be excluded due to similarities in their LPS antigens. The ELISA provides good agreement with characterised samples from quality assessment schemes (IgM 100%, IgG 98%, IgA 95%). ▶

## Immunoblot

Immunoblot detection of anti-*C. trachomatis* antibodies is most effective when three categories of antigen are used: Chlamydia cross-reactive (genus-specific) LPS, highly specific antigens from elementary bodies of *C. trachomatis* and highly specific recombinant MOMP antigen. This composition is provided uniquely by the Anti-Chlamydia trachomatis EUROLINE-WB (Figure 2). The broad antigen spectrum ensures high sensitivity and specificity due to separate detection and combined interpretation of specific and cross-reacting antibodies. The EUROLINE-WB yields a very good agreement (IgG 98%, IgA 97%) with results from precharacterised sera in quality assurance schemes.

The EUROLINE-WB offers the additional advantage of complete automatability from sample identification to final results using the EUROBlotOne device. The specialised software EUROLineScan provides qualitative, separate appraisal of antibodies against the three antigen groups (Figure 3).

## Perspectives

*C. pneumoniae* is amongst the most important human respiratory pathogens, due to its high prevalence, tendency to frequently reinfect and potential to cause serious chronic diseases. It is estimated to be one of the three most frequent etiologic agents of community-acquired pneumonia. Elderly persons and patients with co-morbidities are particularly susceptible to a severe course. Hence, the disease burden of *C. pneumoniae* is likely to increase as the population ages.

*C. trachomatis* is, in contrast, a major concern in the young, healthy population. It is estimated that 5-10% of sexually active young people in Europe and the USA are harbouring a hidden *C. trachomatis* infection. Many of these infections are only identified at later stages when, for example, reproductive problems have already occurred. Screening programmes have been introduced in many countries to help combat this silent epidemic. Alongside direct detection, serology plays an important role in confirmatory laboratory diagnostics and is, moreover, useful for epidemiological studies. It is hoped that increased application of laboratory screening will help to stem the rising Chlamydia tide. **ML**

Figure 1. Anti-Chlamydia MIF

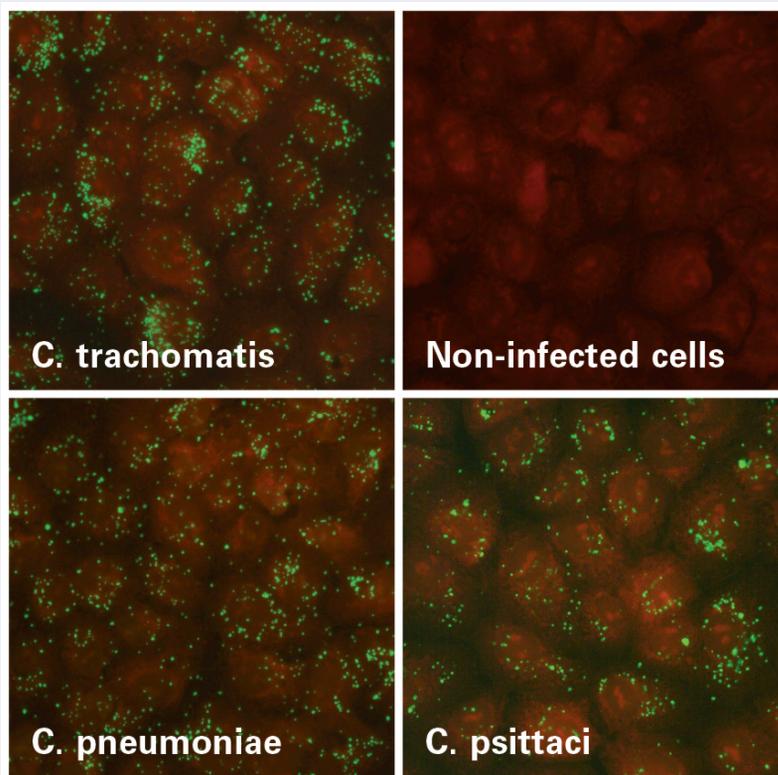


Figure 2. Anti-*C. trachomatis* immunoblot with three categories of antigen

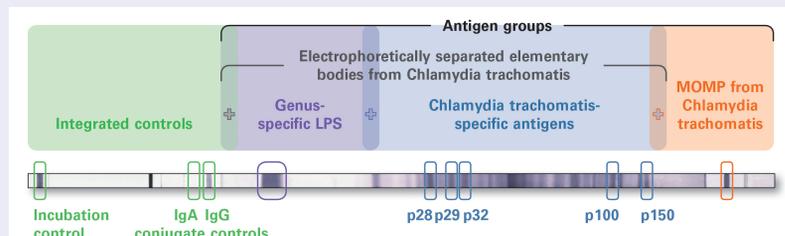


Figure 3. Evaluation of immunoblot results

