

Chlamydia (*Chlamydia trachomatis*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *chlamydia trachomatis*.

Authorisation: PHLN

1 PHLN SUMMARY LABORATORY DEFINITION

1.1 Condition

Chlamydia

1.1.1 Definitive Criteria

- Isolation of *Chlamydia trachomatis* from cell culture inoculated with a genital tract specimen as demonstrated by specific staining; OR
- Detection of *C. trachomatis* by nucleic acid test (NAT) on a genital tract or urine specimen; OR
- Detection of *C. trachomatis* antigen in urethral or endocervical columnar epithelial cells stained with fluorescein-labelled monoclonal antibodies; OR
- Detection of *Chlamydia* (lipopolysaccharide, LPS) antigen in cervical and urethral specimens or in urine samples from men, with confirmation by second method such as a blocking assay or direct fluorescent antibody (DFA); OR
- Detection of *C. trachomatis* ribosomal ribonucleic acid (RNA) by hybridisation with a chemiluminescent DNA probe in endocervical, urethral or (in men) urine samples.

1.1.2 Suggestive Criteria

- Nil.

2 INTRODUCTION

Chlamydia trachomatis are small, non-motile, obligate intracellular bacteria that typically infect human eukaryotic columnar epithelial cells. *C. trachomatis* infections result in a number of diseases of public

health concern worldwide, including trachoma (serovars A-C), lymphogranuloma venereum (LGV serovars L1-3) and urogenital infections (serovars D-K). Chlamydia is the most common sexually transmitted bacterial pathogen worldwide and in Australia has exhibited a steady rise in prevalence¹. National notification rates of newly diagnosed chlamydia infections have increased nearly 4-fold since 1994, more than doubling since 1999.

A plausible reason for the rise in Australian *C. trachomatis* notifications may be an increase in the levels of testing, as a consequence of more readily available, rapid, sensitive and non-invasive tests utilising nucleic acid amplification testing (NAAT)². However, it is likely that this increase is not only due to increased testing, as the percentage increase in rates of chlamydia notifications in Australia far exceeds those of annual Medicare test numbers³.

Paramount in control of *C. trachomatis* infection is prompt recognition and appropriate treatment of an infected individual with simultaneous management of their partner(s). Even when screening is incorporated into control strategies, lack of access to appropriate services (especially in rural and remote areas), reluctance of at-risk populations to attend for screening and treatment, fear of invasive genital examinations, and lower sensitivities of older conventional diagnostic assays reduces the effectiveness of such programmes. Therefore, accurate, cost-effective, reliable diagnostic assays are needed to impact on the incidence of chlamydia. With the advent of NAAT, including target and signal amplification methods, diagnosis of *C. trachomatis* and other sexually transmitted infections (STIs) have been revolutionised and have allowed the use of self-collected non-invasive sampling techniques. Most studies evaluating self-sampling with molecular diagnostic techniques have demonstrated an equivalent or superior detection of chlamydia, as compared to conventional clinician sampling and conventional detection methods⁴⁻¹⁶.

3 LABORATORY BASED TESTS

3.1 Cell Culture

Cell culture was considered the “gold standard” for *C. trachomatis* detection before the development of NAAT¹⁷. Culture is performed by inoculating a confluent monolayer of human epithelial cells and incubating for 48–72 hours, when the infected cells develop characteristic intracytoplasmic inclusions that contain *C. trachomatis* elementary and reticulate bodies. These inclusions are detected by staining with fluorescent-conjugated monoclonal antibody, specific for the major outer membrane protein (MOMP) of *C. trachomatis*^{17, 18}. This approach is labour intensive and technically demanding. In addition it detects viable bacteria which require stringent transport criteria. These limitations, along with lower sensitivity (50–85%) has resulted in cell culture to only be used in cases where the results will be used as evidence in legal investigations such as sexual abuse where high specificity (99–100%) is required. However the 2006 guidelines for treatment of sexually transmitted diseases from the US Centers for Disease control (CDC) suggest that NAAT might be an alternative if culture systems for *C. trachomatis* are unavailable. Then, NAAT confirmation tests should consist of a second FDA-cleared nucleic acid amplification test that targets a different sequence from the initial test¹⁹.

3.1.1 Suitable specimens

Cervical, urethral, rectal, pharyngeal and conjunctival swabs collected and placed in chlamydia transport medium.

3.1.2 Test sensitivity

As this method has conventionally been considered the gold standard, the calculations of 50–85% sensitivity have been estimated when compared to the more sensitive DNA amplification methods¹⁸.

3.1.3 Test specificity

High specificity of 99–100% is attributed to this test.

3.1.4 Suitable Quality Assurance Programs

None available

3.2 Direct Fluorescent Antibody (DFA) test

This antigen detection method is used for rapid direct examination of specimen. It can be utilised as an independent test or to confirm results of another assay. DFA requires cellular material collected by a swab or an endocervical brush, rolled over a slide, fixed and stained with monoclonal antibody specific for MOMP of *C. trachomatis*. Visualisation of more than 10 stained elementary bodies under fluorescence microscopy is generally accepted as a positive result. An important benefit of utilising DFA is that it allows assessment of adequacy of sampling as indicated by visualisation of columnar cells. Although processing of the slide is quite rapid and relatively easy, laborious microscopic examination requires experienced personnel.²⁰

DFA may suit laboratories with small numbers of request and/or where confirmation of another test such as Enzyme Immunoassay (EIA) is required.

3.2.1 Suitable specimens

Cervical, urethral, rectal, pharyngeal or conjunctival swabs collected and placed in chlamydia transport media.

3.2.2 Test sensitivity

Sensitivity, relative to culture, is lower i.e. 80–90%, which makes this test unsuitable in regions where the prevalence of chlamydia is less than 5%.²¹

3.2.3 Test specificity

Relative to culture, DFA has high specificity (98–99%), as it relies on visualisation of stained chlamydial elementary bodies.

3.2.4 Suitable Quality Assurance Programs

RCPA QAP

3.3 Enzyme Immunoassay (EIA)

This assay is based on detection of chlamydial LPS antigen by using an enzyme-labeled antibody that recognises LPS common to all species of chlamydia present in the specimen. LPS is used as it provides for a more abundant soluble target. Generally after binding of the LPS specific antibody, an enzyme converts a colourless or fluorescence-generating substrate to a coloured or fluorescence

product which is detected by a spectrophotometer or a fluorometer. This assay generally can be done in a manual format, in 4 hours, by most laboratories. Because of possible cross reaction with LPS of other bacteria and hence production of false positives, all positive results will either need to be confirmed by blocking assays i.e. addition of monoclonal chlamydial LPS which results in reduction of signal in positive specimens, or an alternative assay such as DFA. Several rapid point-of-care (POC) tests have been developed utilising EIA technology in membrane capture or latex immunodiffusion format. They do not require sophisticated equipment and can be completed in 30 minutes. These POC tests generally have been shown to be less sensitive and specific than laboratory performed EIA, i.e. sensitivity of 52% and specificity of 95%²².

3.3.1 Suitable specimens

Cervical, urethral, rectal, pharyngeal and conjunctival swabs collected and placed in chlamydia transport medium. Urine specimens can also be utilised.

3.3.2 Test sensitivity

Overall the sensitivity and specificity reported for this assay vary quite significantly depending on which method it is compared to. However when compared to culture, sensitivity of 86% has been described²³.

3.3.3 Test specificity

Relative to culture, specificity of 95% has been described, especially if combined with blocking assays²³.

3.3.4 Suitable Quality Assurance Programs

RCPA QAP.

3.4 Serological test

Serological test have not been considered useful in diagnosis of recent chlamydial infection as acute and convalescent sera are required to detect a seroconversion. Even then due to high background rates of past infection, it can be difficult to interpret serological responses. Moreover, whilst the presence of an IgM response may be seen in deep complicated infections such as pelvic inflammatory disease, LGV or infants with chlamydial pneumonitis,¹⁸ they are not useful or present often in genital infections. This test has limited value therefore for detection of genital infection.

3.5 Nucleic acid detection assays

These assays have the advantage that they do not require intact or viable organisms and therefore less stringent transport conditions can be applied. In addition, the turnaround time for results is much shorter compared to culture and a single sample can be used for detection of other targets such as *N. gonorrhoeae*. There are two classes of assays utilised, i.e. nucleic acid hybridization and target amplification assays.

3.5.1 Nucleic acid hybridization assays

These types of assays utilise detection of specific DNA probe complementary to *C. trachomatis* sequences present in specimens. There are currently two FDA approved commercial assays, i.e. Gen-Probe PACE 2 (San Diego, CA, USA) and Digene Hybrid Capture (HC) II (Gaithersburg, MD, USA) assays. The PACE 2 assay utilises a chemiluminescent DNA probe specific

to chlamydial 16S rRNA. The DNA/RNA hybrid is absorbed onto a magnetic bead and signal detected with a luminometer. As chlamydia contain up to 10 000 copies of rRNA, PACE 2 is more sensitive than antigen detection systems ²⁴. The HC II assay utilises an RNA probe complementary to genomic specific RNA probes which after hybridization, antibody capture and signal amplification allows identification of samples containing chlamydial sequences. The RNA probes are homologous to the entire chlamydia cryptic plasmid sequence (7 500 bp) and approximately 39 000 bp of the *C. trachomatis* genome (4%). There are approximately 10 copies of the cryptic plasmid sequence per organism and a single copy of the remaining genomic sequences per organism.

Comparison of PACE 2 with HC II on 1,746 patients from two centres, showed HC II being substantially more sensitive than the PACE 2 test²⁵. Another study considered the test comparable to PCR for sensitivity and specificity²⁶. A multi-centre study on cervical specimens found HC II had a sensitivity of 97.7% and specificity of 98.2% against culture for *C. trachomatis*.

3.5.2 Nucleic Acid Amplification tests (NAAT)

These assays are extremely sensitive and able to detect single target copy and are highly specific. With these qualities these assays can be used in low prevalence and asymptomatic populations and in particular in self-collected samples which may not be directly from site of infection, i.e. urine, vulval swabs.

Several commercial assays, each utilising different amplification technology has FDA approval:

Manufacturer/assay	Technology
Genprobe - APTIMA assay TM	Transcription Medited Amplification (TMA)
BioMerieux - NucliSens TM	Nucleic Acid Sequence Based Amplifaction (NASBA)
BD - ProbeTec TM	Strand displacement Amplification (SDA)
Abbott - LCx TM	Ligase Chain Reaction (LCR)
Roche - AMPLICOR TM	Polymerase Chain Reacton - (PCR)
Abbott - m2000rt TM	Polymerase Chain Reaction - (PCR)

“Aptima, Nuclisens and ProbeTec” assays are isothermal amplification methods and “LCx, Amplicor and m2000rt” are non-isothermal amplification. These assays have been well evaluated for both urogenital and urine specimens showing very high sensitivity and specificity of 90–99% and 100% respectively¹⁶. All these assays can be considered as standard technique for detection of *C. trachomatis* in clinical specimens. Recent studies have shown that these assays can also be utilised for other extra genital specimens, such as pharyngeal and rectal swabs²⁷.

The Amplicor assay is the most utilised across Australian laboratories²⁸. This assay utilises polymerase chain reaction (PCR) to amplify a 207 bp segment of the cryptic plasmid DNA present in *C. trachomatis* strains. Recently a variant strain containing a 377 base pair deletion of the cryptic plasmid was identified in Sweden²⁹. However these mutants have not been found in other parts of

Europe in great numbers and also not been detected yet in Australia,³⁰ although laboratories will need to be vigilant to detect possible potential mutations that may affect sensitivity and specificity of assays in use.

NAATs have also been utilised in detection of more invasive lymphogranuloma venerum (LGV) strains which have recently been shown to become prevalent (in particular L2b genotype) in men who have sex with men in Europe and recently in Australia^{31, 32}. All the NAATs described above are able to detect LGV strains: however in order to differentiate the L2b strain, a real-time PCR assay describes necessary³¹.

3.5.2.1 Suitable specimens

All sample types can be utilised including cervical, urethral, rectal, pharyngeal, conjunctival swabs and/or urine specimens.

3.5.2.2 Test sensitivity

As these assays are the most sensitive of all the diagnostic assays, with a range from 90–100% being reported depending on the assay utilised and gold-standard used for comparison. In general an expanded gold standard has been used, i.e. results from 2 or more amplification assay is used as reference test.

3.5.2.3 Test specificity

Overall specificity of 99–100% has been described¹⁸.

3.5.2.4 Suitable internal controls

Inclusion of an internal control is of utmost importance as it will allow one to rule out inhibition in samples which give negative results.

3.5.2.5 Suitable Quality Assurance Programs

RCPA and NRL both provide excellent programs which are easy to obtain and incorporate in any NAAT assay.

3.5.2.6 Special considerations

As molecular based assays are very sensitive and prone to contamination in the clinic, during transport or in the laboratory, extreme care must be put in place to avoid such incidences. Laboratories conducting such tests should have in place steps to avoid sample-to-sample and PCR amplicon contamination. In addition, it must be appreciated that most molecular assays detect presence of an organism's DNA and hence may not reflect an active infection. This is in particular important when a test of cure is being conducted: a positive result less than 3 weeks after treatment may detect nucleic acid from non-viable organisms and not necessarily mean a new infection or persistent infection.

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