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

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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 prevalence[1]. 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 numbers[3].

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 methods [4-16].

3 LABORATORY BASED TESTS

3.1 Cell Culture

Cell culture was considered the “gold standard” for *C. trachomatis* detection before the development of NAAT[17]. 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 test[19].

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 methods[18].

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 described[23].

3.3.3 Test specificity

Relative to culture, specificity of 95% has been described, especially if combined with blocking assays[23].

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 test[25]. Another study considered the test comparable to PCR for sensitivity and specificity[26]. 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:

Manufactorer/assay	Technology
Genprobe - APTIMA assay ™	Transcription Medited Amplification (TMA)
BioMerieux - NucliSens ™	Nucleic Acid Sequence Based Amplifaction (NASBA)
BD - ProbeTec ™	Strand displacement Amplication (SDA)
Abbott - LCx™	Ligase Chain Reaction (LCR)
Roche - AMPLICOR ™	Polymerase Chain Reacton - (PCR)
Abbott - m2000rt ™	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% respectively[16]. 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 swabs[27].

The Amplicor assay is the most utilised across Australian laboratories[28]. 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 Sweden[29]. 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 necessary[31].

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 described[18].

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.

4 REFERENCES

1. Communicable Diseases Network Australia: National Notifiable Diseases Surveillance System (NNDSS), *NCHECR2005 Annual Surveillance report*.
2. Centres for Disease Control & Prevention Summary of Notifiable Diseases – United States, 2001. *MMWR* 2003; 50:1-108.
3. Chen MY, Fairley CK & Donovan B. Nowhere near the point of diminishing returns: correlations between chlamydia testing and notification rates in New South Wales. *Aust NZ J Public Health* 2005; 29:249-53.
4. Chernesky M, Castriciano S, Sellors J, Stewart I, Cunningham I, Landis S, Seidelman W, Grant L, Devlin C & Mahony J. Detection of *Chlamydia trachomatis* antigens in urine as an alternative to swabs and cultures. *J Infect Dis*. 1990; 161:124-6.
5. Chernesky MA, Jang D, Lee H, Burczak JD, Hu H, Sellors J, Tomazic-Allen SJ & Mahony JB. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. *J Clin Microbiol*. 1994; 32:2682-5.
6. Østergaard L, Moller JK, Andersen B & Olesen F. Diagnosis of urogenital *Chlamydia trachomatis* infection in women based on mailed samples obtained at home: multipractice comparative study. *BMJ* 1996; 313:1186-9.
7. Alary M, Poulin C, Bouchard C, Fortier M, Murray G, Gingras S, Aube M & Morin C. Evaluation of a modified sanitary napkin as a sample self-collection device for the detection of genital chlamydial infection in women. *J Clin Microbiol* 2001;39:2508-12.
8. Garrow SC, Smith DW & Harnett GB. The diagnosis of chlamydia, gonorrhoea, and trichomonas infections by self obtained low vaginal swabs, in remote northern Australian clinical practice. *Sex Transm Infect* 2002; 78:278-81.
9. Knox J, Tabrizi SN, Miller P, Petoumenos K, Law M, Chen S & Garland SM. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. *Sex Transm Dis* 2002;
29:647-54.
10. Wiesenfeld HC, Heine RP, Rideout A, Macio I, DiBiasi F & Sweet RL. Vaginal introitus: a novel site for *Chlamydia trachomatis* testing in women. *Am J Obstet Gynecol* 1996; 174:1542-6.
11. Schachter J, McCormack WM, Chernesky MA, Martin DH, Van Der Pol B, Rice PA, Hook EW, Stamm WE, Quinn TC & Chow JM. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis. J Clin Microbiol*. 2003; 41:3784-9.
12. Tabrizi SN, Chen S, Fairley C, Borg AJ, Migliorini G, Lees M & Garland SM. Tampon-collected genital cells in detection of *Chlamydia trachomatis* by polymerase chain reaction. *J Infect Dis*. 1993; 168:796-797.
13. Tabrizi SN, Paterson B, Fairley CK, Bowden FJ & Garland, SM. A self-administered technique for the detection of sexually transmitted diseases in remote communities. *J Infect Dis*. 1997;176:289-292.
14. Tabrizi SN, Paterson BA, Fairley CK, Bowden FJ & Garland SM. Comparison of tampon and urine as self-administered methods of specimen collection in the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis* in women. *Int J STD AIDS* 1998;9:347-349.
15. Tabrizi SN, Fairley CK, Chen S, Giouzeppos O, Paterson B, Bowden FJ, Garland SM. Evaluation of patient-administered tampon specimen for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Sex Trans Dis* 2000; 27:133-7.
16. Garland SM, Tabrizi SN, Hallo J, Chen S. Assessment of *Chlamydia trachomatis* prevalence by PCR and LCR in women presenting for termination of pregnancy. Sex Transm Infect. 2000;76:173-176
17. Lees MI, Newnan DM. Garland SM. Simplified culture procedure for large-scale screening for Chlamydia trachomatis infections. *J Clin Microbiol*. 1988;26:1428-1430
18. Black CM. Current Methods of Laboratory Diagnosis of *Chlamydia trachomatis* infections. *Clinical*

Micriobiology Reviews 1997;10:160-184.
19. Centers for Disease Control and Prevention, Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. MMWR 2006; 55:1-94.
20. Lees MI, Newnan DM, Garland SM. Detection of *Chlamydia trachomatis* by direct immunofluorescence and culture in genital specimens of symptomatic and asymptomatic women. Venereology. 1990;3:28-31.
21. Sackett DL, Haynes RB, Tugwell P. Clinical epidemiology, 1985; p. 59–138. Little, Brown & Co., Boston.
22. Lauderdale TL, Landers L, Thorneycroft I, Chapin K. Comparison of the PACE 2 assay, two amplification assays, and Clearview EIA for detection of *Chlamydia trachomatis* in female endocervical and urine specimens. *J Clin Microbiol*. 1999;37:2223-9.
23. Moncada J, Schachter J, Shafer MA, Williams E, Gourlay L, Lavin B, Bolan G. Detection of *Chlamydia trachomatis* in first catch urine samples from symptomatic and asymptomatic males. Sex Transm Dis. 1994;21:8-12.
24. Lees MI, Newnan DM, Garland SM. Comparison of a DNA probe assay with culture for the detection of *Chlamydia trachomatis*. J Med Microbiol. 1991;35:159-161
25. Modarress KJ, Cullen AP, Jaffurs WJ Sr, Troutman GL, Mousavi N, Hubbard RA, Henderson S, Lorincz AT. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in swab specimens by the Hybrid Capture II and PACE 2 nucleic acid probe tests. *Sexually Transmitted Diseases* 1999;26:303 - 308.
26. Girdner JL, Cullen AP, Salama TG, He L, Lorincz A, Quinn TC. Evaluation of the Digene Hybrid Capture II CT-ID test for detection of *Chlamydia trachomatis* in endocervical specimens. *Journal of Clinical Microbiology* 1999; 37:1579-81.
27. Lister NA, Tabrizi SN, Fairley CK, Garland SM. Validation of Roche COBAS Amplicor assay for detection of *Chlamydia trachomatis* in rectal and pharyngeal specimens by an *omp1* PCR assay. *J. Clin Micro* 2004;42: 239-41.
28. Land S, Tabrizi SN, Gust A, Johnson E, Garland S, Dax EM. External Quality Assessment Program for Chlamydia trachomatis Diagnostic Testing by Nucleic Acid Amplification Assays. *J. Clin Microbiol*. 2002;40:2893-6.
29. Ripa T, Nilsson P. A variant of *Chlamydia trachomatis* with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill.
2006;11. http://www.eurosurveillance.org/ew/2006/061109.asp#2.
30. Stevens MP, Tan SE, Horvath L, Fairley CK, Garland SM, Tabrizi SN.. Absense of a *Chlamydia trachomatis* variant, harbouring a deletion in the cryptic plasmid, in clients of a sexually transmissible infection clinic and antenatal patients in Melbourne. *Communicable Disease Intelligence*. 2008; 32:77-8.
31. Spaargaren JH, Fennema S, Morre SA, de Vries HJ, Coutinho RA. New lymphogranuloma venereum *Chlamydia trachomatis* variant, Amsterdam. *Emerg. Infect. Dis*. 1005; 11:1090-2.
32. Stark D, van Hal S, Hillman R, Harkness J, Marriott D. Lymphogranuloma venereum in Australia: anorectal *Chlamydia trachomatis* serovar L2b in men who have sex with men. *J Clin Microbiol*. 2007;45:1029-31.