***Mycoplasma genitalium***

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 *mycoplasma genitalium*.

**Authorisation:**  PHLN

**Consensus date:**  17 October 2019

1 PHLN Summary Laboratory Definition

1.1 Condition:

*Mycoplasma genitalium*

1.1.1 Definitive Laboratory Criteria

Detection of *Mycoplasma genitalium* DNA by species-specific target by nucleic acid amplification test (NAAT) from an appropriate clinical specimen

1.1.2 Suggestive Criteria

Nil

2 Introduction

The Organism

*M. genitalium* is a member of the Mollicutes class of bacteria and the Mycoplasmataceae family. It is a fastidious obligate intracellular bacterium, and measuring approximately 0.6 × 0.3 μm is the smallest prokaryote capable of self-replication. *M. genitalium* lacks a cell wall and a result is not visible on Gram stain. Genome size is 580kb, and contains 482 protein-coding genes. Culture is extremely difficult; as such it is not routinely performed in diagnostic laboratories. Growth is optimal at 370C in anaerobic conditions with 5% CO2. Cultures should be incubated for 1-2 months, with colonies having a fried-egg appearance1.

Clinical features

*M. genitalium* infection is associated with male non-gonococcal urethritis (NGU) and non-chlamydial male non-gonococcal urethritis (NCNGU)2. *M. genitalium*was first isolated from men with non-gonococcal urethritis (NGU) in 19813, and has subsequently been detected in rectal infections, including prostatitis, and balanitis / balanoposthitis.Infection in women is associated with vaginal discharge, urethritis, cervicitis, pelvic inflammatory disease4, preterm delivery and spontaneous abortion). Although it has been linked to tubal factor infertility, the data remains inconclusive. Rare cases of extra-genital infections have been reported, including arthritis.).

Since the development of *M. genitalium* specific nucleic acid amplification tests (NATs) the organism has been increasing recognised as a sexually transmitted infection. Prevalence in young adults (18-27 years old) is estimated to be approximately 1% (1.1% males, 0.8% females)7. Prevalence is as high as 15-25% in men with symptomatic NGU and up to 15% of women with PID)8. Asymptomatic infection was detected in 9.5% of men who have sex with men (MSM) in Melbourne. Rectal positivity was higher than urine (7.0% v 2.7%), and co-infection with other STIs was found in 17% 9. In this Australian study 84% of isolates were macrolide resistant.

Early studies identified azithromycin had superior efficacy compared with doxycycline, the standard therapy for NGU, with a clinical efficacy of 85% compared to 30-40%4, leading to guidelines recommending azithromycin as first line therapy for *M. genitalium* infections. Recently, antimicrobial resistance in *M. genitalium* has emerged as a significant public health issue, with 30- 84% of Australian isolates9, 11, 12 and 40-60% of *M. genitalium* strains in male NGU strains world-wide being macrolide resistant. A Queensland study found azithromycin resistance rates of 62% respectively, with differing resistance rates according to region, site of sample and sex. The highest resistance rates were seen in rectal swab samples from males, and southeast Queensland12. Macrolide resistance is associated with point mutations in region 5 (V-region) of the 23S rRNA gene. A number of macrolide resistance mutations (MRMs) have been identified, including single nucleotide polymorphisms (SNPs) at positions 2059, 2058 and 2062 (Escherichia coli numbering)13, 14.

Resistance to the fluoroquinolone class is also emerging with 10%12 of Queensland isolates being ciprofloxacin resistant. A study from a Victorian Sexual Health clinic detected fluroquinolone resistance mutations *parC* and *gyrA* in 13.6% and 5% of patients respectively, with a significant association between the presence of parC S83 mutations and clinical failure11. These reports have resulting in management recommendations incorporating resistance testings into the treatment algorithm15.

*M. genitalium* is not notifiable in Australia, and as a result there is no NNDSS data available.

3 LaboratoryDiagnosis

3.1 Culture

Although*M. genitalium* may be cultured in highly specialised laboratories, culture is not a routine diagnostic method.

3.2 Serology

Serological assays have been developed for the detection of *M. genitalium,*and include micro-immunofluorescence, immunoblotting and enzyme immunoassays. Serological diagnosis is confounded by antigenic variability and cross-reaction with other Mycoplasma infections8. To date no serological assay has been standardised or commercialised, and as a result serology does not have a role in routine diagnostic testing.

3.3 Nucleic Acid Amplification Tests (NAAT)

3.3.1 Assays for the detection of *M. genitalium*

Nucleic acid testing for the detection of *M. genitalium* was first developed in 1991, and remains the only appropriate diagnostic method. A number of molecular targets have been used for diagnostic assays (Table 1), both alone and in multiplex assays that detect additional urogenital pathogens. Assays targeting the MgPa operon offer optimal sensitivity. A number of commercial assays have been developed (Table 2), although the majority are currently not TGA approved.

No data is available regarding the optimal time after exposure to testing, however a 2 week period is considered the minimal incubation period. The Australian Contact Tracing Guidelines recommend test of cure, to be performed at least 2 weeks after treatment is completed (4 weeks after commencement of therapy)16.

**Table 1: Range of assay targets for the detection of *M. genitalium***

|  |  |  |
| --- | --- | --- |
| **Target** | **Analytical sensitivity** | **Reference** |
| MgPa operon (adhesion protein gene) | < 5 genome copies | 17 |
| housekeeping gene *gap*(glyceraldehyde-3-phosphate dehydrogenase (G3PDH) | 5 genome copies | 18 |
| 16S rRNA gene | 10 genome copies | 19 |
| mg219 gene | 825 genome copies (0.5 pg) | 20 |
| mgpB gene | 5 genome copies | 21 |
| pdhG gene (dihydrolipoaminde dehydrogenase) | 10 genome copies | 22 |

**Table 2: Commercial assays for the detection of *M. genitalium*24, 25**

|  |  |  |  |
| --- | --- | --- | --- |
| **Assay** | **Manufacturer** | **Target** | **Method and Detection** |
| Aptima *Mycoplasma genitalium* assay | GeneProbe/Hologic | 16S rRNA | PCR, target capture, transcription-mediated amplification (TMA), and hybridization protection assay (HPA) |
| ResistancePlus MG | SpeeDx Pty Ltd. | *MgPa* gene 23S rRNA gene (macrolide resistance) | MG PlexZyme and PlexPrime technology Multiplex, real-time quantitative PCR |
| LightMix | TIB Molbiol, Roche Diagnostics | *gap* gene | Monoplex real-time PCR |
| S-DiaMGTV | Diagenode | *mg219*gene | Multiplex real-time quantitative PCR |
| STDetect Chip | Lab Genomics, Seongnam |  | PCR microarray |
| Amplisens *N gonorrhoeae / C.trachomatis/M.genitalium/ T.vaginalis*MULTIPRIME FRT | Interlab Science |  | Multiplex real-time PCR with hybridisation-fluorescence detection |
| Bio-Rad Dx CT/NG/MG assay | Bio-Rad | *MgPa* gene | Multiplex PCR |

3.3.2 Assays for the detection of *M. genitalium* antimicrobial resistance

With the emergence of high levels of macrolide resistance, resistance-guided sequential treatment of *M. genitalium* infections has entered clinical practice26. The majority of assays that detect resistance mutations are in house assays, with detection by sequencing, labelled hydrolysis probes, high-resolution melting analysis, and melt curve analysis27. In general these methods are less sensitive compared to the standard *M. genitalium* detection quantitative PCR (qPCR). The SpeeDx Resistance Plus MG assay (SpeeDx, Sydney, Australia) is available in Australia and TGA registered for urine and swab samples. It simultaneously targets *M. genitalium* (MgPa target) and five 23S rRNA mutations28. AusDiagnostics (Mascot, Australia) produce a number of Tandem-Plex Urinogenital and Resistance panels that incorporate detection of *M. genitalium*, macrolide and fluoroquinolone resistance. The Elitech Mg Macrolide qPCR assay is CE-IVD marked for urine with evaluations ongoing for swab samples however is not currently TGA approved.

Suitable specimens

Testing should only be undertaken in symptomatic individuals or contacts of cases.

Optimal specimen type for detection of *M. genitalium* has not been determined. There is moderate variability in sensitivity and specificity between specimen sites and assays. Throat specimens are not recommended due to the low presence of pharyngeal carriage29.

|  |  |
| --- | --- |
| **Specimen** | **PCR sensitivity28** |
| Genital mycoplasma infection (disorder) | 97.4% |
| *Mycoplasma genitalium* (organism) | 74.3% |
| Mycoplasma culture (procedure) | 95.7% |
| Polymerase chain reaction analysis (procedure) | 85.7% |
| *Mycoplasma genitalium* DNA (substance) | 24.3% |

**Swab type:**

Culture swab transport system (Dacron or rayon swab), with or without Stuart or Amies liquid medium Plain swab (Dacron or rayon with aluminium or plastic shaft).

3.4 Quality Assurance

A quality assurance program is available through the RCPA QAP.

4 Agreed Typing & Subtyping Methods

There is no *standard national or international organism or subtyping method.*

4.1 Laboratory Nomenclature for National Database Dictionary

|  |  |
| --- | --- |
| **SNOMED CT concept** | **Code** |
| Genital mycoplasma infection (disorder) | 240594008 |
| *Mycoplasma genitalium* (organism) | 708378006 |
| Mycoplasma culture (procedure) | 104176001 |
| Polymerase chain reaction analysis (procedure) | 9718006 |
| *Mycoplasma genitalium* DNA (substance) | 708378006 |
| Polymerase chain reaction analysis for genomic fingerprinting (procedure) | 252370006 |

5 References

1. McGowin CL and Totten PA. The Unique Microbiology and Molecular Pathogenesis of *Mycoplasma genitalium. J Infect Dis* 2017;216(S2):S382–8.
2. Jensen JS. *Mycoplasma genitalium*: the aetiological agent of urethritis and other sexually transmitted diseases. *J Eur Acad Dermat Venereol* 2004;18:1-11.
3. Tully JG, Taylor Robinson D, Cole RM, Rose DL. A newly discovered mycoplasma in the human urogenital tract. *Lancet* 1981;1:1288-1291.
4. Jensen JS, Cusini M, Gomberg M, Moi H. Background review for the 2016 European guideline on *Mycoplasma genitalium* infections. *J Eur Acad Dermat Venereol* 2016;30:1686-1693.
5. Wiesenfeld H and Manhart L. *Mycoplasma genitalium* in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen. *J Infect Dis* 2017:216 (Suppl 2) :S389-395.
6. Taylor Robinson D, Gilroy CB, Horowitz S, Horowitz J. *Mycoplasma genitalium* in the joints of two patients with arthritis. *Eur J Clin Microbiol Infect Dis* 1994;13:1066-9.
7. Manhart LE, Holmes KK, Hughes JP, Housten LS, Totten PA. *Mycoplasma genitalium* among young adults in the United States: An emerging sexually transmitted infection. *Am J Public Health* 2007;97: 1118-1125.
8. Taylor-Robinson D, Jensen J. *Mycoplasma genitalium*: from Chrysalis to multi-coloured butterfly. *Clin Microbiol Rev* 2011;24:498-514.
9. Read TR, Murray GL, Danielewski JA et al. Symptoms, sites and significance of *Mycoplasma genitalium* in men who have sex with men, *EID* 2019;25:719-727.
10. Hughes G, Saunders J. *Mycoplasma genitalium:*the next sexually transmitted superbug? BMJ 2018;363;k4376.
11. Murray GL, Bradshaw CS, Bissessor M et al. Increasing macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*. *EID* 2017;23:809-812.
12. Sweeney EL, Trembizki E, Bletchly C, Bradshaw CS et al. Levels of *Mycoplasma genitalium* Antimicrobial Resistance Differ by Both Region and Gender in the State of Queensland, Australia: Implications for Treatment Guidelines. *J Clin Microbiol* 2019: 27:57(3).
13. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin Treatment Failure in *Mycoplasma genitalium*–Positive Patients with Non-gonococcal Urethritis Is Associated with Induced Macrolide Resistance. *Clin Infect Dis* 2008; 47:1546–53.
14. Chrisment D. Detection of macrolide resistance in *Mycoplasma genitalium* in France. *J Antimicrob Chemother.* 2012 Nov;67(11):2598-601.
15. [Australian Society of HIV Medicine. The Australasian STI Management Guidelines 2018](http://www.sti.guidelines.org.au/).
16. [Australian Contact Tracing Guidelines](http://contacttracing.ashm.org.au/conditions/when-contact-tracing-is-recommended/mycoplasma-genitalium) Accessed May 2019.
17. Jensen, Bjornelius, Dohn, Lidbrink. Use of TaqMan 5 Nuclease Real-Time PCR for Quantitative Detection of *Mycoplasma genitalium*DNA in Urethritis Who Were Attendees at a Sexually Transmitted Disease Clinic. *J Clin Microbiol* 2004; 42;683-92.
18. Svenstrup HE, Jensen JS, Björnelius E et al. Development of a Quantitative Real-Time PCR Assay for Detection of *Mycoplasma genitalium*. *J Clin Microbiol* 2005, 43 (7) 3121-3128. 3
19. Yoshida, T., T. Deguchi, M. Ito, S.I. Maeda, M. Tamaki, and H. Ishiko. 2002. Quantitative detection of *Mycoplasma genitalium* from first-pass urine of men with nongonococcal urethritis and asymptomatic men by real-time PCR. *J. Clin. Microbiol*. 40:1451-1455.
20. Cjalker V, Jordan K, Ali T, Ison C. Real-time PCR detection of the mg219 gene of unknown function of *Mycoplasma genitalium* in men with and without non-gonococcal urethritis and their female partners in England. *J Med Microbiol* 2009;58:898-9.
21. Jensen JS. Protocol for the detection of Mycoplasma genitalium by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol* 2012;903:129-139.
22. Muller EE, Venter JM, Magooa MP et al. Development of a rotor-gene real-time PCR assay for the detection and quantification of Mycoplasma genitalium. [*J Microbiol Methods.*](https://www.ncbi.nlm.nih.gov/pubmed/?term=muller+genitalium+microbiol+methods2012)2012 Feb;88(2):311-5.
23. Munsen E. Molecular Diagnostic Update for the Emerging (If Not Already Widespread) Sexually Transmitted Infection Agent *Mycoplasma genitalium*: Just About *Ready* for Prime Time. *J Clin Microbiol*; 55:2894-2902.
24. Gaydos, C. *Mycoplasma genitalium*: Accurate Diagnosis is Necessary for Adequate Treatment. *J Infect Dis* 2017;216(S2);S406-11.
25. Read T, Fairley C, Murray et al. Outcomes of Resistance-guided Sequential Treatment of *Mycoplasma genitalium* Infections: A Prospective Evaluation. *Clin Infect Dis* 2019; 68:554-60.
26. Braam J, van Marm S, Seevers T et al. Sensitive and specific assay for the simultaneous detection of *Mycoplasma genitalium* and macrolide resistance associated mutations. *Eur J Clin Microbiol Infect Dis* (2018) 37:2137-2144.
27. Tabrizi S, Tan L, Walker S et al. Multiplex assay for simultaneous detection of *Mycoplasma genitalium* and macrolide resistance using PlexZyme and PlexPrime technology. Plos One 2016;11:e0156740.
28. Tabrizi S, Su L, Bradshaw C et al. Prospective Evaluation of ResistancePlus MG, a New Multiplex Quantitative PCR Assay for Detection of *Mycoplasma genitalium*and Macrolide Resistance. *J Clin Microbiol* 55(6):1915–1919.
29. Lillis, RA. Nsuami, M.J Myers, L, Martin, D. Utility of Urine, Vaginal, Cervical, and Rectal Specimens for Detection of *Mycoplasma genitalium* in Women. *J Clin Micro* 2011;49:1990-2.