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*.

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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 appearance[1].

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 1981[3], 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 disease[4], 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 isolates[9, 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 Queensland[12]. 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 failure[11]. These reports have resulting in management recommendations incorporating resistance testings into the treatment algorithm[15].

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 infections[8]. 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 practice[26]. 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 analysis[27]. 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 mutations[28]. 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 carriage[29].

Specimen	PCR sensitivity[28]
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

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