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***Chlamydia trachomatis* and *Neisseria gonorrhoeae* user information**

At Micropathogy Ltd. we offer PCR testing for *Chlamydia trachomatis* (Ct), *Neisseria gonorrhoeae* (Ng) and a duplex assay combining these same targets (CtNg).

Chlamydia trachomatis

Chlamydia trachomatis is a Gram-negative, non-motile, obligate intracellular bacterium which exists outside host cells as inactive elementary bodies but can infect host cells via endocytosis and reside as membrane protected inclusion bodies intracellularly.

There are 15 known serovars and three biovars which cause distinct clinical syndromes:

- Serovars Ab, B1, B2 and C cause chronic follicular keratoconjunctivitis or 'trachoma' which may lead to chronic scarring and the most common cause of preventable blindness worldwide.
- Lymphogranuloma venereum: LGV (serovars L1, L2 and L3) causing an endemic STI in Africa, India, South-East Asia, South America and the Caribbean. More likely in the UK in MSM detected from rectal swabs.
- Serovars D-K cause both common STI infections resulting in urethritis, pelvic inflammatory disease, ectopic pregnancy and infertility, but can also affect neonates born to positive mothers causing pneumonia and conjunctivitis.

Chlamydia is the most commonly diagnosed bacterial STI in the UK. As infection often has no symptoms but can have serious health consequences, the National Chlamydia Screening Programme (NCSP) recommends that Chlamydia screening should be offered as an integrated component of existing sexual and reproductive health services and through a range of other settings such as primary care and internet-based testing to ensure that young people (<25 years) have universal access to testing. From June 2021, NCSP opportunistic testing for the asymptomatic was refocused on women only.

Due to the fastidious nature of this intracellular organism and the requirement for a very high throughput method, NAATs are exclusively the test of choice for detection of *C. trachomatis.* The PCR often targets the cryptic plasmid due to its high copy number making these assays very sensitive.

At Micropathology we target the cryptic plasmid in a nested PCR. Urine, swabs, thin preps (endocervical cellular specimens) and semen are validated sample types although semen is still awaiting UKAS. Other samples may be tested and reported along with an appropriate caveat stating that the sample is not validated for this assay.

Specimens sent for STI screening are typically tested on the CtNg duplex instance due to higher throughput. Any specimens deemed positive or suspected of containing *C. trachomatis* DNA on this duplex are confirmed using a repeat on the Ct (single-plex) assay. Samples received for LGV DNA testing are run on the Ct and CtNg assay to confirm the presence of *Chlamydia trachomatis* DNA and reduce the risk of false negatives LGV typing results.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a Gram-negative intracellular diplococcus that only infects humans and causes the sexually transmitted disease gonorrhoea; the second most common bacterial STI in the UK. Rising rates of antibiotic resistance, the diseases persistence and its association with poor reproductive outcomes has made disease from this organism a major health concern.

Gonorrhoea commonly presents as a purulent disease of the cervix or the urethral mucous membrane. In males, symptoms occur in over 90% of individuals and may be severe, with discharge and/or dysuria appearing two to five days following exposure. Women are less likely to display severe symptoms (~50%)[1], however may report an increased or altered vaginal discharge. In about a quarter of individuals, lower abdominal pain is present. The organisms can also be found often asymptomatically as a rectal or pharyngeal infection through screening and contact tracing.

Secondary local complications (eg. Epididymitis and pelvic inflammatory disease) and metastatic complications (eg. Arthritis) may occur if the primary infection is not adequately treated. Other disseminated infections include endocarditis, skin lesions, painful joints, meningitis, or perihepatic inflammation (Fitz-Hugh-Curtis syndrome). As *N. gonorrhoea*e is always considered a pathogen, where it is detected, gonococcus should always be treated to prevent further complications and onward spread. A test of cure is recommended for all cases due to antibiotic resistance and the seriousness of further infection[1] and contract tracing should be conducted to prevent continued transmission. Infection cannot be ruled out in individuals who test within two weeks of sexual contact with an infected partner; therefore, BASHH guidelines recommended that patients return for repeat testing after this window period if epidemiological treatment is not initially given[1]. Additionally, as approximately 19% of patients with gonorrhoea have concurrent *C. trachomatis* infection, testing for other STIs should be undertaken where gonorrhoea is detected.

A further consideration is ophthalmia neonatorum, the presentation of purulent conjunctivitis/inflammation (sticky eye) of the new-born often within 24 hours to five days post birth after infection through the birth canal. Around 30-50% of neonates born to infected mothers will develop a conjunctivitis of which progression is usually rapid and serious complications such as visual impairment and perforation are not uncommon. Where found this disorder should be reported and treated rapidly and until 2010 was a notifiable disease.

Multiple methods can be used for the diagnosis of gonorrhoea. For immediate near patient results, microscopy can be used to identify intracellular Gram-negative diplococci in purulent material from male swabs. The primary role of culture is now for antimicrobial susceptibility testing, which is of increasing importance as antimicrobial resistance in *N. gonorrhoeae* continues to evolve and spread. All individuals with gonorrhoea diagnosed by NAAT should have cultures taken for susceptibility testing prior to treatment.

Use of NAAT tend to be more sensitive than culture, particularly in oropharyngeal and rectal sites showing high sensitivity (>95%) in both symptomatic and asymptomatic infection[2]. Different tests available provide differing levels of cross-reactivity with other Neisseria and closely related organisms particularly in the pharynx therefore confirmation using from these sites using a different NAAT is recommended. NAATs may also be crucial in diagnosing infection in extra-genital sites such as from joint fluid or heart valves.

The Micropathology assay is a nested PCR targeting the 16S rRNA gene of *Neisseria gonorrhoeae*. Urine, swabs and thin preps (endocervical cellular specimens) and semen are validated sample types although semen is still awaiting UKAS accreditation. Other samples may be tested and reported along with an appropriate caveat stating that the sample is not validated for this assay.

Specimens sent for STI screening are typically tested on the CtNg duplex assay in the first instance due to higher throughput. Any specimens deemed positive or suspected of containing *N. gonorrhoeae* DNA on the duplex are confirmed using a repeat on the Ng (single-plex) assay. Any pharyngeal swabs that are deemed positive or suspected of containing *N. gonorrhoeae* DNA are sent for sequencing for additional confirmation.

Reference

[1]Helen Fifer, John Saunders, Suneeta Soni, S Tariq Sadiq, Mark FitzGerald (2019) British Association for Sexual Health and HIV national guideline for the management of Infection with *Neisseria gonorrhoeae*.

[2]Ng, L.-K., & Martin, I. E. (2005). The laboratory diagnosis of Neisseria gonorrhoeae. *The Canadian Journal of Infectious Diseases & Medical Microbiology*, *16*(1), 15–25.