

## CTNG Suggested Protocol

### CT/NG Clinical Relevance:

**Chlamydia** (*Chlamydia trachomatis*) is the most prevalent sexually transmitted bacterial infection (~3-4 million cases annually). **Gonorrhea** (*Neisseria gonorrhoeae*) is a bacterial infection that often co-exists with Chlamydia (~700,000 cases annually). Infection with either organism can cause pelvic inflammatory disease (PID), ectopic pregnancy, and infertility in women, and testicular and prostate infections, and sterility in men.

CytoGenes offers primer mixes and controls allowing laboratories to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by quantitative PCR. Results are qualitative, indicating the presence or absence of each of the target organisms. An internal control primer set is available to verify the quality of the DNA extraction process.

### Scope of Procedure:

The following is a recommended procedure for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by quantitative PCR. Researchers are advised to utilize this protocol as a guide in the development of their own procedure. This protocol was developed utilizing ancillary reagents and equipment purchased from external vendors. Protocol modifications will be required dependent on the selection of ancillary reagents and equipment utilized in your laboratory.

The following protocol provides instructions for processing samples. Laboratories are encouraged to develop their own guidelines on the selection and use of standards, controls and data analysis.

## CytoGenes Reagents (Required):

Cat #	Item
CTNG-CT	CT Primer Mix
CTNG-NG	NG Primer Mix
CTNG-ICP	Int Con Primer Mix

## CytoGenes Reagents (Optional):

Cat #	Item	Assay Targets Included	Concentration
CTNG-PC	CTNG Positive Control	CT, NG	1e <sup>5</sup> copies/ul
CTNG-ICS	Internal Control DNA Spike	Int Con Primer Mix	2e <sup>3</sup> copies/ul

## Ancillary Reagents (Recommended):

- EXPRESS One-Step Superscript® qRT-PCR (ThermoFisher)

## Equipment and Supplies (Required):

- Quantitative PCR instrument
- PCR plates, tubes and sealing film.
- Pipettors (range 1-1000ul)
- Aerosol barrier pipette tips.
- Microcentrifuge

## Protocol:

1. If utilizing the Internal Control DNA Spike, add 5ul to each sample prior to DNA extraction.
2. Isolate DNA from samples utilizing standard procedures utilized by your laboratory.

- Prepare separate PCR reactions for each of the PCR primer sets to be tested. (Note: for multiple samples, prepare a master mix and scale the volumes appropriately based on the number of samples to be tested).

Reagent	Volume (ul)
EXPRESS SuperScript® qPCR SuperMix	10
CytoGenes 25X PCR Primer Mix	0.8
Nuclease Free Water	4.2
Sample	5
Total volume	20

- Prior to initiating the run, program Q-PCR instrument to detect the probes utilizing the appropriate filter sets. The probes in each of the master mixes are labeled with a FAM reporter and BHQ quencher.
- Initiate PCR protocol appropriate for a Q-PCR reaction. The following is a recommended protocol based on our instruments and ancillary reagents utilized.

Cycle	Step	Temp (°C)	Time (Min:Sec)	Repeats
1	1	95	2:00	1
2	1	95	:15	40
	2	60	:30	

- Analyze results.

## References:

- Jaton K, Bille J, Greub G. A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs. *J Med Microbiol.* 2006 Dec;55(Pt 12):1667-74. PubMed PMID: 17108270.
- Whiley DM, Limnios A, Moon NJ, Gehrig N, Goire N, Hogan T, Lam A, Jacob K, Lambert SB, Nissen MD, Sloots TP. False-negative results using *Neisseria gonorrhoeae* porA pseudogene PCR - a clinical gonococcal isolate with an *N. meningitidis* porA sequence, Australia, March 2011. *Euro Surveill.* 2011 May 26;16(21). pii: 19874. PubMed PMID: 21632019.