

PrimerDesign™ Ltd

Quantification of *Neisseria Gonorrhoeae* genomes

PorA gene

For general laboratory and research use only

Standard Kit





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Introduction to *Neisseria Gonorrhoeae*

Neisseria gonorrhoeae is a Gram-negative coccus of the *Neisseria* genus. This bacterium has a spherical shape with diameter of 0.6-1µm and is usually seen in pairs infecting human cells. The species has a circular DNA genome of approximately 1Mbp encoding over 2000 genes. The fimbriae of this bacterium are several micrometers in length and are essential for host cell adhesion. *N. gonorrhoeae* is capable of motility via fimbriae extension, adhesion and contraction.

N. gonorrhoeae is transmitted by sexual contact and usually causes infection in cells of the mucous membrane of the male urethra or the endocervix and urethra in females. The bacteria attach to the non-ciliated host columnar, epithelial cells and enter the cell by endocytosis, mediated by the major porin protein, Por. The endocytosed vacuoles are transported to the base of the cell where the bacteria are released to replicate at the basement membrane. During infection, polysaccharides are released from the bacteria that stimulate host cell production of tumour necrosis factors that cause an inflammatory response. This inflammation draws neutrophils to the infection site to clear the bacteria by phagocytosis. However, many bacteria can survive within the phagocyte until they are released upon neutrophil cell death. One polysaccharide in particular, lipooligosaccharide (LOS), is also known to cause damage to the mucosal membrane indirectly by promoting the release of proteases within the host cell. There is no vaccine against *N. gonorrhoeae* infection and antibiotic resistance is beginning to increase. Therefore, treatment is by a course of antibiotics that will be effective against resistant strains and also treat possible co-infection with Chlamydia.

After a 2-3 day incubation period in males, a purulent discharge from the urethra is noticeable as well as the development of dysuria. These symptoms can be seen in around 95% of cases of male infection. Women infected with the bacterium are less likely to be symptomatic, although when symptoms do develop these can be non-specific and can be confused for other infections; these symptoms can include vaginal discharge, dysuria, and abdominal pain.

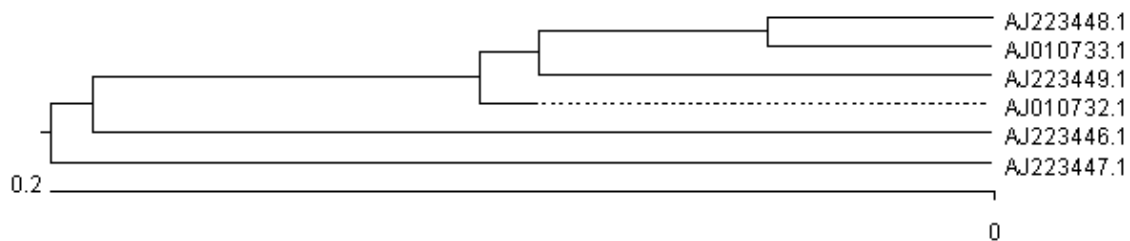
N. gonorrhoeae infections can cause complications when the bacterium enters the blood or other cells. Complications in males caused by the infection can result in prostatitis or orchitis if the bacteria spread. In females, invasion of the fallopian tubes or ovaries can result in salpingitis or ovaritis respectively, with any of these infections possibly resulting in sterility.



Specificity

The *PrimerDesign™ Quantification Kit for Neisseria Gonorrhoeae* Genomes is designed for the *in vitro* quantification of *Neisseria Gonorrhoeae* isolates. The primers have 100% homology with all PorA sequences in the phylogenetic tree below and therefore have the broadest detection profile. The primers have a low homology to the closely related *Neisseria meningitides* and will not detect on this strain.

Fig.1 *Neisseria Gonorrhoeae* PorA Sequences detected





Kit Contents

- Pathogen specific primer/probe mix (150 reactions **BROWN**)
- Pathogen positive control template (for Standard curve **RED**)
- RNase/DNase free water

Reagents and equipment to be supplied by the user

- Real-Time PCR Instrument
- **Mastermix or Mastermix components**
This kit is designed to work well with all commercially available Mastermixes. However, we recommend the use of PrimerDesign 2x Precision™ Mastermix.
- **Pipettors and Tips**
- **Vortex and centrifuge**
- **Thin walled 1.5 ml PCR reaction tubes**

Kit storage

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilized components have been re-suspended unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for six months from date of purchase.



Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity. Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions PrimerDesign pathogen detection kits have very high priming efficiencies of >95% and can detect between 1×10^8 and 1×10^2 copies of target template.



Principles of the test

Real-Time PCR

A pathogen specific primer and probe mix is provided and the target sequence can be detected through the **FAM** channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the pathogen DNA/cDNA. A fluorogenic probe, is included in the same reaction mixture which consists of an oligonucleotide labeled with a 5`-reporter dye and a downstream, 3`-quencher, During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real time PCR platforms.

Positive control

For copy number determination, and as a positive control for the PCR set up, the kit contains positive control template. This can be used to generate a standard curve of pathogen copy number / CT value. Alternatively the positive control can be used at a single dilution for a qualitative analysis of the samples. Each time the kit is used, at least one positive control reaction must be included on the run. A positive result indicates that the primers and probes for quantification of the target pathogen gene are working properly in your particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false positive results. This can be achieved by handling this component in a Post PCR environment.

Negative control

To confirm absence of contamination, a negative control reaction should be included every time the kit is used. For this reaction, the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources or contamination should first be explored and removed.



Carry-over prevention using UNG (optional)

Carry over contamination between PCR reactions can be prevented by including uracil-N-glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. PrimerDesign recommend the application of 0.2 U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step (95°C for 10 minutes).



Bench side Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Barrier tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening.

This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.

2. Reconstitute the kit components according to the table below

To ensure complete reconstitution, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use.

Component	Volume
Pre-PCR box	
Primer/Probe mix (BROWN)	165 µl
Post-PCR bottle	
Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.



Real-time PCR detection

1. Prepare a reaction mix according to the table below

Include sufficient reactions for the standard curve wells (8 samples in duplicate) and also the negative control.

Pathogen detection mix

Component	1 reaction
2X Precision™ Mastermix	10 µl
Pathogen primer/probe mix (BROWN)	1.0 µl
RNAse/DNAse Free water	4.0 µl
Final volume	15 µl

2. Pipette 15µl of this mix into each well according to your real-time PCR experimental plate set up.

3. Prepare sample DNA templates for each of your samples (suggested concentration 5ng/µl) in RNAse/DNAse free water.

If the concentration of DNA is not known, then dilute your DNA sample reactions 1:20 (10 µl of sample DNA and 190µl of water)

4. Pipette 5µl of diluted template into each well, according to your experimental plate set up.

For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl



5. Preparation of standard curve dilution series

- 1) Pipette 900 μ l of RNase/DNase free water into 7 tubes and label 2-8
- 2) Pipette 100 μ l of Positive Control Template (**RED**) into tube 2
- 3) Vortex thoroughly
- 4) Change pipette tip and pipette 100 μ l from tube 2 into tube 3
- 5) Vortex thoroughly

Repeat steps 4 and 5 to complete the dilution series

Standard Curve	Copy Number
Tube 1 Positive control (RED)	2×10^7 per μ l
Tube 2	2×10^6 per μ l
Tube 3	2×10^5 per μ l
Tube 4	2×10^4 per μ l
Tube 5	2×10^3 per μ l
Tube 6	2×10^2 per μ l
Tube 7	20 per μ l
Tube 8	2 per μ l

6. Pipette 5 μ l of standard template into each well, according to your experimental plate set up.

The final volume in each well is 20 μ l.



Amplification Protocol

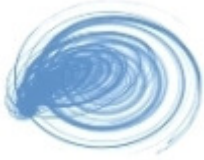
Amplification conditions using PrimerDesign 2X Precision™ MasterMix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37°C
	Enzyme Activation (if required)***	10 mins	95°C
50 cycles	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

* Fluorogenic data for the control DNA should be collected during this step through the FAM channel

** Required if your mastermix includes UNG to prevent PCR carryover contamination

*** Not all Mastermixes require this enzyme activation step. Follow the manufactures instructions for your mastermix.



Notices and disclaimers

During the warranty period PrimerDesign pathogen detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

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