

Primerdesign™ Ltd

C.trachomatis, N.gonorrhoeae & T.pallidum

genesig® PLEX kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction

C.trachomatis

Chlamydia trachomatis is an obligate, coccoid or rod-shaped bacterium that is an intracellular pathogen of humans. It is classed as a Gram-negative bacterium although it lacks a peptidoglycan cell wall. Its genome is circular double-stranded DNA, around 1,042K nucleotides in length with an extrachromosomal plasmid of about 7.5Kbp of which there are 7 -10 copies per bacterial particle. *C.trachomatis* causes Chlamydia, the most common sexually transmitted disease in the world and Trachoma, a common cause of preventable blindness among children in developing nations. *C.trachomatis* cannot survive outside of the eukaryotic cell and is transmitted through infected bodily secretions containing the elementary body form of the bacterium. Chlamydia infections are often asymptomatic but in some cases after an incubation time of one to three weeks, symptoms in males can be seen. These include pain during urination, discharge from the penis and pain, infection or inflammation of the testicles or testicular ducts. Delay or lack of treatment can result in pelvic inflammatory disease (PID) in women and infertility in both sexes.

N.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. *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. 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 non-resistant strains and treat possible co-infection with Chlamydia.

After a 2-3day 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 with other infections.

T.pallidum

Treponema pallidum is a Gram-negative bacterium of the Spirochaetes phylum. The bacteria are approximately 6-20µm in length with a helical shape. An endoflagella found in the periplasmic space between the outer and inner membrane enable corkscrew motility. The bacterial genome is one double stranded DNA circular chromosome of around 1Mbp in length that codes for over 1000 proteins. This bacterium is the parasite responsible for causing Syphilis and is also thought to increase the likelihood of transmission of HIV.

T. pallidum acquires all of its energy through glycolysis but relies on a host for nutrients and therefore cannot survive in the absence of the host cells. The initial site of infection is the epithelial cells of the genitals, although the bacterium can spread throughout the body to any tissue or organ. The infection is a sexually transmitted disease and is spread by contact with skin lesions or exposed mucus membranes. However, congenital syphilis is transmitted from mother to fetus. Previous infections with *T. pallidum* offer no immunity, but the infection can be treated with antibiotics such as penicillin which it has not shown resistance to. *T. pallidum* infection progresses through 4 stages if untreated: primary, secondary, latent and tertiary. Primary infection presents in a chancre lesion at the site where the bacteria penetrates the epithelial cell. These indurated ulcers are clean and painless and can last up to 5 weeks. If left untreated the second stage of the infection follows with a rash appearing approximately 6 weeks after the initial infection, accompanied by skin lesions. People infected may also experience tiredness, fever, headaches, sore throat, loss of appetite and swollen glands which can last up to 6 weeks. Several months later symptoms disappear although the bacterium is still present, this is the latent phase where transmission rates decrease significantly. If the infection is not treated, the tertiary phase may begin up to several years later. At this stage, syphilis can affect the bones, the cardiovascular system and the central nervous system causing disease.

Specificity

The genesigPLEX kit is designed for the in vitro detection of *C.trachomatis*, *N.gonorrhoeae* and *T.pallidum*.

The kit is designed to have a broad detection profile. Specifically, the primers represent 100% homology with over 95% of the NCBI database reference sequences available at the time of design.

The dynamics of genetic variation means that new sequence information may become available after the initial design. Primerdesign periodically reviews the detection profiles of our kits and when required releases new versions.

If you require further information or have a specific question about the detection profile of this kit, then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit contents

- Multiplex primer/probe mix (100 reactions **BROWN**)
FAM, VIC, ROX and Cy5 labelled (see table below)

Target	Fluorophore
C.trachomatis (CT)	FAM
N.gonorrhoeae (NG)	VIC
T.pallidum (TP)	ROX
Endogenous control	Cy5

- Multiplex positive control template (**RED**)
- Lyophilised oasis™ PLEX Master Mix (**BLUE**)
- oasis™ resuspension buffer (**BLUE**)
- Template preparation buffer (**YELLOW**)
for resuspension of positive control template
- RNase/DNase free water (**WHITE**)
for resuspension of primer/probe mix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

DNA extraction kit

This kit is recommended for use with genesig EASY DNA/RNA Extraction kit. However, it is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

Pipettors and tips

Vortex and centrifuge

Thin walled 1.5ml tubes

qPCR plates

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances. Primerdesign does not recommend using the kit after the expiry date stated on the pack.

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 DNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions genesig kits have very high priming efficiencies of >90% and can detect between 1×10^8 and 1×10^2 copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig 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. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc. and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

Primerdesign™ is a trademark of Primerdesign Ltd.

genesig® is a registered trademark of Primerdesign Ltd.

oasig™ is a trademark of Primerdesign Ltd.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applied Biosystems Corporation. BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Primerdesign™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.

Principles of the test

Real-time PCR

Individual primer and probes designed for each target have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target cDNA. Fluorogenic probes are included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 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 qPCR platforms.

Positive control

For a positive control, the kit contains a single positive control that contains templates for the three targets in the test. The kit positive control will give a *C.trachomatis* signal through the FAM channel, an *N.gonorrhoeae* signal through the VIC channel and a *T.pallidum* signal through the ROX channel. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting each virus are working properly in that particular run. 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 these components in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To confirm the 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.

Endogenous control

To confirm extraction of a valid biological template, the single primer/probe mix supplied contains primers and probe designed to detect an endogenous gene. Detection of the endogenous control is through the Cy5 channel. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimise 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. Filter 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. Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:
To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in water	Volume
Pre-PCR pack	
Multiplex primer/probe mix (BROWN)	110µl

3. Resuspend the positive control template in the template preparation buffer supplied, according to the table below:
To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive control template (RED)*	500µl

* 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.

4. Resuspend the lyophilised oasigPLEX Master Mix in oasig resuspension buffer, according to the table below:

Component – resuspend in oasig resuspension buffer	Volume
Lyophilised oasigPLEX Master Mix (BLUE)	525µl

DNA extraction

1. Complete the DNA extraction according to the manufacturer's recommended protocols.

qPCR detection protocol

1. For each DNA sample prepare a reaction mix according to the table below: Include sufficient reactions for positive and negative controls.

Component	Volume
oasigPLEX Master Mix (BLUE)	10µl
Multiplex primer/probe mix (BROWN)	1µl
RNase/DNase free water (WHITE)	4µl
Final volume	15µl

2. Pipette 15µl of these mixes into each well according to your qPCR experimental plate set up.
3. Pipette 5µl of DNA sample 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.
4. Pipette 5µl of positive control template into each well according to your plate set up.
The positive control contains template for *C.trachomatis*, *N.gonorrhoeae* and *T.pallidum*. The final volume in each well is 20µl.

qPCR amplification protocol

Amplification conditions using oasigPLEX Master Mix

	Step	Time	Temp
	Enzyme activation	2 mins	95°C
Cycling x 50	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

* Fluorogenic data should be collected during this step through the FAM, VIC, ROX and Cy5 channels.

Interpretation of results

Positive control

The positive control well should give an amplification plot through the FAM channel (CT), the VIC channel (NG) and the ROX channel (TP). There is no endogenous control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each virus.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

Endogenous control

The signal obtained from the endogenous control reaction will vary according to the amount of biological material present in each sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.

Sample data

Presence of the viruses are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those viruses. It may be possible for samples to contain multiple viruses, therefore positive results the FAM, VIC and ROX channels may be present.

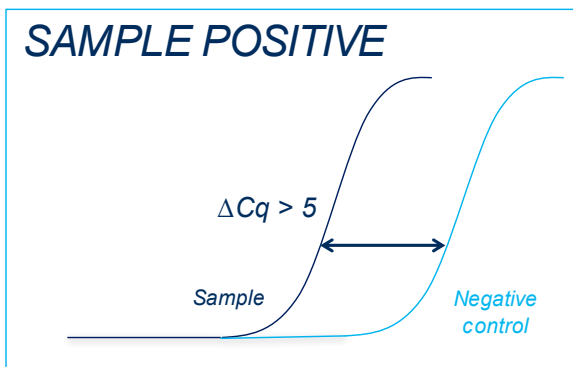
Summary of data interpretation

Target (FAM/VIC)	Endogenous control (Cy5)	Positive Control	Negative Control	Interpretation
FAM +	+ / -	+	-	CT POSITIVE RESULT
VIC +	+ / -	+	-	NG POSITIVE RESULT
ROX +	+ / -	+	-	TP POSITIVE RESULT
-	+	+	-	NEGATIVE RESULT

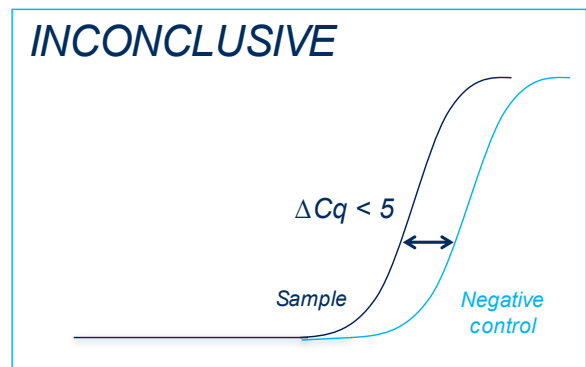
+ / -	+ / -	+	≤35	EXPERIMENT FAILED Due to test contamination
+ / -	+ / -	+	>35	*
-	-	+	-	SAMPLE PREPARATION FAILED
+ / -	+ / -	-	+ / -	EXPERIMENT FAILED

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

* Where the test sample is positive, and the negative control is positive with a Cq >35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.