# *Presto* combined qualitative real time CT/NG assay

CE 0344 Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices

IVD **For In Vitro Diagnostic Use Only**

## Test Instructions

**Combined *Chlamydia trachomatis-Neisseria gonorrhoeae* real time amplification DNA assay**

Real time amplification DNA assay for the qualitative *in vitro* detection of *Chlamydia trachomatis (CT)* and *Neisseria gonorrhoeae (NG)* DNA in urine, urethral swab and endocervical swab specimen.

**Catalogue Number: CG 160100, 4 x 25 tests**
**Catalogue Number: CG 160500, 5 x 100 tests**

Store at **-20°C** upon receipt

## Contents

## 1. Intended use

The Goffin Molecular Technologies Presto *Chlamydia trachomatis* (CT)- *Neisseria gonorrhoeae* (NG) Assay kit is intended to be used for *in vitro* qualitative detection of *Chlamydia trachomatis* plasmid DNA and *Neisseria gonorrhoeae* chromosomal DNA in urine, urethral swab specimen and endocervical swab specimen of human origin.

The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. No special training will be required for routine laboratories performing quantitative PCR.

## 2. Summary and explanation of the test

*Chlamydia trachomatis* is an obligate intracellular gram-negative bacterium. Fifteen different serovars are known including eight (D-K) causing urogenital infections. Infection in females is frequently asymptomatic, and untreated infections may progress to endometritis, salpingitis and infertility. Screening for asymptomatic infections is indicated to reduce the transmission of CT and to prevent the development of severe complications[1].

*Neisseria gonorrhoeae* is responsible for the second most prevalent sexually transmitted disease after *Chlamydia trachomatis*. Infection in females is frequently asymptomatic which increases the risk of transmission. Long term undiagnosed infections may progress to pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, neonatal conjunctivitis, and infertility. Early detection of infection is indicated to reduce the transmission and subsequent severe complications[2]

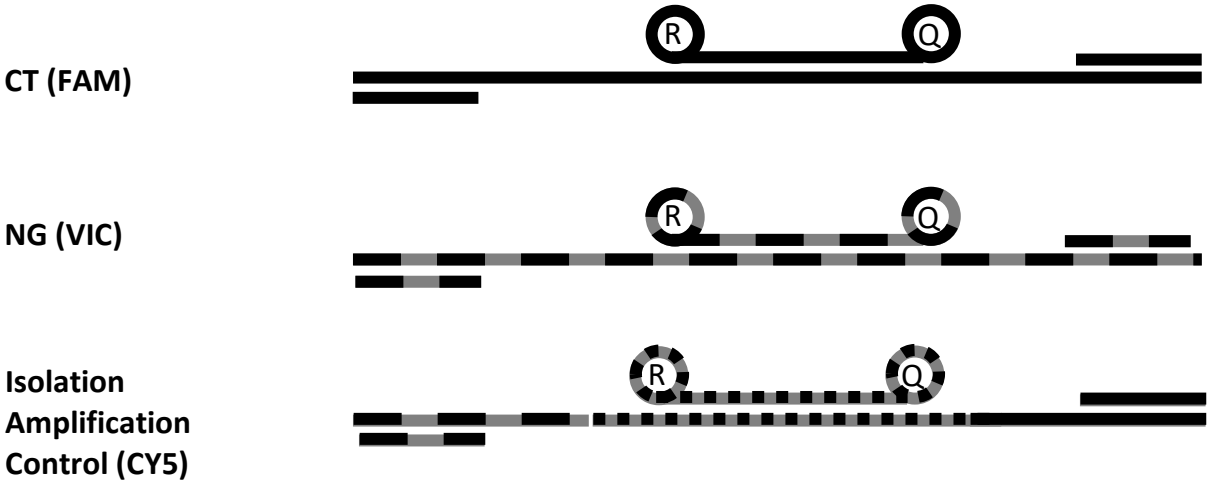
Real time PCR has proven to be a sensitive and easy to use diagnostic tool for many micro-organisms and is a standard technique to detect CT/NG. It is known that there are specimen specific but unknown inhibitory substances present in some clinical samples, which are not always reliably been removed during sample preparation. A specific designed isolation/inhibition Isolation Amplification Control is introduced which will recognize inefficient DNA isolation and/or PCR inhibition in each individual clinical sample.

Combining real time PCR with the addition of an Isolation Amplification Control, which monitors both inhibition and nucleic acid extraction of clinical samples, generates the best of two worlds. The Presto CT/NG kit provides this combination and warrants a correct interpretation omitting false negative and producing true positive signals with high sensitivity and specificity. The real time format of the kit reduces the risk of contamination by amplicons. In addition, the *Chlamydia trachomatis* Swedish variant strain will be successfully detected. Finally, another unique feature is introduced in the Presto kit. To circumvent negative results in double infections with one prominent (highly positive) bacterium, CT and NG selectors are available. These selectors will allow detection of low load of one target in combination with high load of the other target in the same sample.

## 3. Principle of the test procedure

The detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) DNA by the polymerase chain reaction (PCR) is based on the amplification of a part of the CT cryptic plasmid DNA and the opa genes of NG using specific oligonucleotides. The PCR products are detected by internal probes, each linked with a different fluorescent reporter dye (“R”). The fluorescence of the reporter is quenched by a quencher group (“Q”) linked to the same probe. During formation of the PCR product, the probe is degraded and fluorescence of the reporter is no longer quenched and can be detected in real time. An Isolation Amplification Control bacterium is added before isolation and amplification of a clinical specimen. This control bacterium contains a DNA fragment with the reverse primer recognition site for CT

and the forward primer recognition site for NG. The amplified sequence between the primers however is different from the CT/NG fragments. This is detected by a probe sequence with a different reporter dye. The fluorescence of the IAC reporter dye can be used to monitor both isolation and amplification efficiency.




## Selectors

The introduction of selectors introduces a new concept in real time detection of double infections. The kit provides a CT as well as a NG selector. These selectors are meant to select for either CT or NG in a clinical sample highly positive (IAC negative) for NG or CT preventing the risk of a false negative double infection. The use of selectors is advised when a sample is positive for CT or NG. The function of the selector will restore the IAC signal so that even weak positive CT or NG signals can be detected in the presence of high loads of NG or CT DNA, respectively. For this purpose the same isolated DNA fraction can be used in a subsequent PCR while adding the selector of choice before the amplification. For high CT positive samples the NG selector should be added and for high positive NG positive samples the CT selector should be added before amplification (see protocol for volumes).

## 4. Reagents

**PRECAUTIONS**

**CAUTION: Handle patient samples as Biohazardous material.**
**Handle samples as if capable of transmitting an infectious agent.**

All clinical samples should be regarded as infectious. These samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious specimen in the Centre for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

Wear protective gloves

Use sterile aerosol resistant pipette tips

A uni-directional workflow must be adhered to in the laboratory with different rooms for sample preparation, pre-amplification area and post-amplification.

**NOTE:** Reagents in this assay contain **sodium azide** as a preservative (0.05%). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drains with generous amounts of cold water to prevent azide build-up.

### 4.1.1 Components in each Presto CT/NG CG 160100, 4 x 25 assays/ kit

1. **CT/NG PCR mix MASTER MIX** (CG301001)
   4 tubes with a black colour code, labelled ”MASTER MIX” containing 400 µl ready to use PCR mix. This mixture contains four primers for amplification of the CT DNA, NG DNA and the Isolation Amplification Control DNA. It contains three fluorescent probes for detection of CT DNA, NG DNA and control DNA respectively. The master mix contains all ingredients for PCR amplification including DNA polymerase.

2. **CT/NG Isolation Amplification Control Isolation Amplification Control (CG301003)**.
   4 tubes with a red colour code, labelled ”Isolation Amplification Control” containing 150 µl Isolation Amplification Control. The Isolation Amplification Control consists of an inactivated *E. coli* modified with a genomic DNA fragment containing primer binding sites identical to the *C. trachomatis* and *N. gonorrhoeae* sequences, but with a different intermediate probe sequence. NOTE: resuspend before use.

3. **CT Positive Control Positive Control CT** (CG301005)
   4 tubes with a blue colour code, labelled “Positive Control CT” containing 55 µl positive control. The positive control consists of CT DNA at 4 IFU/ 10 µl.

4. **NG Positive Control Positive Control NG** (CG301006)
   4 tubes with a green colour code, labelled ”Positive Control NG” containing 55 µl positive control. The positive control consists of NG DNA at 100 CFU/ 10 µl.

5. **CT/NG Negative Control** **Negative Control** (CG301014)
2 tubes with a transparent colour code, labelled ”Negative Control” containing 500 µl negative control.

6. **CT Selector** **CT SELECTOR** (CG301008)
1 tube with a violet colour code, labelled ”CT SELECTOR” containing 50 µl of CT selector for use with high NG positive samples for the detection of a weak positive CT-co-infection.

7. **NG Selector** **NG SELECTOR** (CG301007)
1 tube with a yellow colour code, labelled ‘NG SELECTOR’ containing 50 µl of NG selector for use with high CT positive samples for the detection of a weak positive NG-co-infection.

Note: Use all components of the same kit lot number.

### 4.1.2 Components in each Presto CT/NG CG 160500, 5 x 100 assays/ kit

2. **CT/NG PCR mix** **MASTER MIX** (CG301015)
5 tubes with a black colour code, labelled ”MASTER MIX” containing 1600 µl ready to use PCR mix. This mixture contains four primers for amplification of the CT DNA, NG DNA and the Isolation Amplification Control DNA. It contains three fluorescent probes for detection of CT DNA, NG DNA and control DNA respectively. The master mix contains all ingredients for PCR amplification including DNA polymerase.

2. **CT/NG Isolation Amplification Control** **Isolation Amplification Control (CG301016)**.
5 tubes with a red colour code, labelled ”Isolation Amplification Control” containing 750 µl Isolation Amplification Control. The Isolation Amplification Control consists of an inactivated *E. coli* modified with a genomic DNA fragment containing primer binding sites identical to the *C. trachomatis* and *N. gonorrhoeae* sequences, but with a different intermediate probe sequence. NOTE: resuspend before use.

3. **CT Positive Control** **Positive Control CT** (CG301017)
4 tubes with a blue colour code, labelled “Positive Control CT” containing 300 µl positive control. The positive control consists of CT DNA at 4 IFU/ 10 µl.

4. **NG Positive Control** **Positive Control NG** (CG301018)
4 tubes with a green colour code, labelled ”Positive Control NG” containing 300 µl positive control. The positive control consists of NG DNA at 100 CFU/ 10 µl.

5. **CT/NG Negative Control** **Negative Control** (CG301014)
2 tubes with a transparent colour code, labelled ”Negative Control” containing 500 µl negative control.

6. **CT Selector** **CT SELECTOR** (CG301008)
1 tube with a violet colour code, labelled ”CT SELECTOR” containing 50 µl of CT selector for use with high NG positive samples for the detection of a weak positive CT-co-infection.

7. **NG Selector** **NG SELECTOR** (CG301007)
1 tube with a yellow colour code, labelled ‘NG SELECTOR’ containing 50 µl of NG selector for use with high CT positive samples for the detection of a weak positive NG-co-infection.

Note: Use all components of the same kit lot number.

## 4.2 Additional materials and instruments required

The test can be used with standard guanidine iso-thiocyanate based lysis reagents and magnetic beads capture devices for DNA isolation.

PCR amplification can be carried out with any real time PCR apparatus able to detect the fluorophores: FAM, VIC, ROX & Cy5. Small variations in cut off values however may occur due to differences in DNA isolation and/or PCR amplification detection depending on the apparatus used. In these cases a new cut off value can be determined without loss of sensitivity and specificity.
Note: No specific software is required for any calculation of the ROX signal. The ROX signal is included to make the test applicable for ABI 7500 instruments (and any other instruments that needs ROX). Software of all other real-time PCR instruments can use the Presto kit without limitations (e.g. LightCyclers).

Use sterile DNase-free polypropylene disposables for all steps in the procedure.

## 4.3 Reagent preparation and storage

1. **CT/NG PCR mix** **MASTER MIX** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

2. **CT/NG Isolation Amplification Control** **Isolation Amplification Control** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

3. **CT Positive Control** **Positive Control CT** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

4. **NG Positive Control** **Positive Control NG** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

5. **Negative Control** **Negative Control** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

6. **CT Selector** **CT SELECTOR** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

7. **NG Selector** **NG SELECTOR** Thaw the tube prior to opening. Opened vial can be refrozen and thawed again only twice. Remark: mix and spin down in a short 3 seconds centrifugation before use.

Store all components at -20 °C.

All components are temperature sensitive. Thaw only the components that are going to be used. Components can be refrozen twice. Keep kit reagents at 2 – 8 °C (on ice) when in use. Store the components at 2 – 8 °C for no longer than 4 days.

## 4.4 Chemical or physical indications of instability

Alteration in the physical appearance of test kit materials may indicate instability or deterioration. Expiry dates shown on component labels indicate the date beyond which components should not be used.

## 5. Specimen collection and preparation of samples

The Presto CT/NG kit is intended to be used on endocervical and urethral swab specimens and urine specimens. General collection devices can be used for standard DNA isolation procedures according to the manufacturer's protocols.

**CAUTION: Handle patient samples as Biohazardous material. Handle samples as if capable of transmitting an infectious agent.**

**Example swab specimen**

1. Collect endocervical and urethral swab specimens and store them in 2-5 ml of 2SP transport medium.

2. Use only validated swab devices. Do not use wooden or aluminium swabs for molecular detection.

3. Keep the swabs in the transport medium. Refrigerate (2 – 8 °C) or freeze swab specimens that will not be processed immediately. Specimens can be stored for 7 days at 2 – 8 °C.

**Example urine specimen**

PATIENT SHOULD NOT HAVE URINATED DURING 2 HOURS PRIOR TO SAMPLE COLLECTION

1. Collect 10 to 30 ml of first catch urine into a clean polypropylene container without any preservatives.

2. Follow the laboratories collection and transport procedures. Refrigerate (2-8 °C) urine specimens that will not be processed immediately. Specimens can be stored for 7 days at this temperature.

**Specimen Preparation**

This procedure must be performed in the Specimen Preparation Area in a class 2 Biological Safety Cabinet (protection to user and material).

**DNA Isolated specimen:**
This kit was validated with use of Nucleic Acid extracted samples by means of BioMérieux NucliSENS® easyMAG™. Please follow the manufacturer's instructions for a description of the system features, isolation protocols and operational guidelines.

Swabs: 200 µl vortexed specimen + 5 µl resuspended IAC + 2 ml easyMAG lysis buffer and elution in 60 µl of which 10 µl is used in the CT/NG PCR.

Urine: 500 µl vortexed urine + 5 µl resuspended IAC + 2 ml easyMAG lysis and elution in 60 µl of which 10 µl is used in the CT/NG PCR.

## 6. Presto CT/NG assay Kit Test Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use aerosol barrier tips during the whole test procedure.

### 6.1 Reagent Preparation

Thaw the mixes needed and keep them at 2-8°C.

### 6.2 Procedure

1. Prepare the required number of reaction tubes or wells for the number of specimens to be measured, plus two tubes for the positive controls, one tube for the negative isolation control and one tube for the negative control.

2. Add 15 µl of the master mix to each reaction to be measured.

3. Vortex and spin down all DNA extracts. Carefully open specimen containers one by one and avoid contamination of gloves and pipette. Using a new aerosol barrier tip for each extract, add 10 µl DNA (Chapter 5) to the reaction tube/well containing the master mix. Replace gloves if suspect of contamination.

4. Using a new aerosol barrier tip, add 10 µl of each positive control Positive Control to the designated reaction tubes/wells containing the master mix. Carefully open the container and avoid contamination of gloves and pipette. Replace gloves if suspect of contamination.

5. Using a new aerosol barrier tip, add 10 µl of the negative control Negative Control to the designated reaction tube/well containing the master mix.

6. Close the reaction tubes or seal the plate, spin down and move the plate to the Amplification Area.

7. Load the reaction tubes/plate into the ABI PRISM® 7500 SDS*. Program the PCR System with following settings:

| | |
|---|---|
| Fixed threshold: | 0.01 |
| Activation polymerase: | 30 seconds 95 °C |
| Number of cycles: | 40 cycles |
| Denaturation: | 3 seconds 95°C |
| Annealing, extension and exonuclease activity: | 30 seconds 60°C |
| Manual baseline settings | |

*For Roche LightCycler480 II choose detection format “3 Colour Hydrolysis Probe”, when another format is used most likely a colour compensation has to be performed once according to the manufacturer protocol.

### Use of Selectors

In case of a positive signal with either CT or NG with $C_{t/p}$<21 (e.g. 14-21) the PCR must be repeated with 1 µl of either of the selectors and 9 µl of isolated DNA: for CT positive samples use NG selector; for NG positive samples use CT selector. Amplification conditions are as described above. By adding the selector a new signal will only appear in case of a double infection. The signal of the primary detected STD is always stronger as compared to the secondary identified STD. A weak signal for the primary positive target may still be present. In a study on 12.254 samples in a STD clinic almost 50% more double infections were detected. However, since only 2.1% double infections were detected among all CT/NG positives after the use of selectors, one can also use the selectors only in case of high risk patients like men who have sex with men (MSM) and swingers. This will reduce the number of repeated test needed.

## 6.3 Procedural notes

1. Use a uni-directional workflow in the laboratory.

   ***Specimen Preparation area:*** Dedicated area to prepare the samples. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area. Materials from this area may not be moved to the Pre-Amplification area.
   ***Pre-Amplification area:*** Dedicated area to prepare the reagents. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area.
   ***Amplification area:*** Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area. Materials from this area, may not be moved to the Pre-Amplification Area, and may not be moved to the Specimen Preparation Area.

2. Always use aerosol resistant tips.

3. Be extremely careful when handling materials to prevent contamination. Always mix and spin down reagents and samples before opening. In case of any suspect of contamination, discard the materials.

4. Discard all consumed reagents upon completion of procedure in compliance with local biohazardous waste regulations.

5. Careful analytical techniques and strict adherence to the directions in the Test Instructions are essential to obtain reliable results.

6. Samples with equivocal results must be verified by repeat assays or isolation.

7. Do not pool reagents from different lots.

8. If the kit is damaged upon receipt, please contact your local distributor and/or Goffin Molecular Technologies BV.

## 7. Interpretation of results

The following dyes are used for the different targets:

| Target | Dye |
|---|---|
| CT | FAM |
| NG | VIC |
| IC | CY5 |

For valid runs (Valid Positive and Negative Control – See Quality Control), interpret the specimen results as follows:

| $C_T$ Specimen | $C_T$ Internal Control | Interpretation |
|---|---|---|
| > 40 | ≤ 37 | ***C. trachomatis* and/or *N. gonorrhoeae* DNA not detected**.<br>This does not necessarily indicate absence of *C. trachomatis* and/or *N. gonorrhoeae* infection, since this depends on correct specimen collection. |
| ≤ 35 for CT **or** NG | ANY | ***C. trachomatis* or *N. gonorrhoeae* DNA detected**.*<br>Depending on the signal *C. trachomatis* DNA or *N. gonorrhoeae* DNA present. To evaluate the presence of the other pathogen, the PCR can be repeated with 1 µl of either of the selectors (see § 6.2, Use of Selectors). CT positive: use NG selector; NG positive: use CT selector. The IAC signal will confirm proper functioning of the selector and the absence of inhibition. In case of a double infection an additional signal for the other target will be visible. |
| ≤ 35 for CT **and** NG | ANY | ***C. trachomatis* and *N. gonorrhoeae* DNA detected**.* |
| > 40 | > 37 | **Inhibitory Specimen**.<br>No diagnosis can be established. Process the original specimen again or process a newly collected specimen. |
| > 35, < 40 | < 40 | **Equivocal.**<br>Conclusions cannot be drawn in respect to *C. trachomatis* DNA and/or *N. gonorrhoeae* DNA. When the detected DNA can be confirmed by repeat analysis, the specimen can be regarded as *C. trachomatis* and/or *N. gonorrhoeae* DNA detected. |

*DNA detection may indicate a CT and/or NG infection but specimen may contain target DNA, without the presence of any living organisms.

### Quality control

Two positive controls and one negative control are provided in each kit for quality control purposes. Additional controls may be analysed in addition to those provided. Established statistical methods for analysing control values and trends should be employed.

If the controls do not comply with the established limits and repetition excludes errors in technique, check the following areas:

1. Expiration date on reagent package and prepared reagents
2. Temperature of the reagents
3. Settings PCR System
4. Contamination

If controls are still invalid, please contact Goffin Molecular Technologies customer service or your local distributor.

**Negative Control**
The $C_T$ of the Negative control should be >40. If the $C_T$ is lower, the whole run is invalid and the test procedure must be repeated.

**Positive CT Control**
The $C_T$ of the Positive CT control should be ≤ 37. If the $C_T$ is outside this range, the whole run is invalid and the test procedure must be repeated.

**Positive NG Control**
The $C_T$ of the Positive NG control should be ≤ 37. If the $C_T$ is outside this range, the whole run is invalid and the test procedure must be repeated.

**Isolation Amplification Control**
The $C_T$ of the Isolation Amplification Control should be ≤ 37. If the $C_T$ is > 37, the sample is invalid and must be repeated.

**Negative Isolation Control**
For each isolation series a negative isolation control to which IAC is added must be analysed. The IAC $C_T$ of the Negative control should be ≤ 37. For CT and NG the $C_T$ should be > 40. If the $C_T$ is lower, all specimens are invalid and the test procedure must be repeated.

## 8. Limitations of the procedure

1. Use only endocervical swab, urethral swab and urine specimens. Other specimen types have not been validated and may result in false positive or false negative results.

2. Specimen collection, transport and storage may affect the number of organisms present in the specimen, causing a false positive or a false negative result.

3. Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination of reagents and/or specimens.

4. Plasmid-free *C. trachomatis* is not detected.

5. The user should have a laboratory education in PCR techniques or have gained appropriate experience in the field of PCR techniques.

## 9. Performance characteristics

**Analytical specificity**

The analytical specificity of the PRESTO CT/NG Test was tested against 37 bacteria, 5 yeast, 1 protozoa and 4 viral strains that may be isolated from the urogenital tract. Each isolate was tested at a concentration of at least $10^4$ copies/test in the absence and presence of CT ($C_T$ around 30) or NG (equimolar mix of 2 distinct NG strains; $C_T$ around 30). All organisms tested (listed below), including 11 non-*N. gonorrhoeae* Neisseria species gave negative results by the PRESTO CT/NG Test and showed no interference with CT and NG detection.

*Actinomyces israelii*
*Bacteroides fragilis*
*Branhamella catarrhalis*
*Chlamydophyla pneumoniae*
*Chlamydoplyla psittaci*
*Candida albicans*
*Candida glabrata*
*Candida krusei*
*Candida parapsilosis*
*Candida tropicalis*
*Citrobacter freundii*
*Clostridium perfringens*
*Cryptococcus neoformans*
Cytomegalovirus
*Enterobacter cloacae*
*Enterococcus faecalis*
*Enterococcus faecium*
Epstein-Barr Virus
*Escherichia coli*
*Gardnerella vaginalis*
*Haemophilus influenzae*
Herpes simplex virus 1
Herpes simplex virus 2
*Klebsiella pneumoniae*
*Lactobacillus species*
*Legionella pneumophila*
*Morganella morganii*
*Mycoplasma pneumoniae*
*Neisseria cinerea*
*Neisseria elongata*
*Neisseria flavescens*
*Neisseria lactamica*
*Neisseria meningitidis*
*Neisseria mucosa*
*Neisseria perflava*
*Neisseria polysaccharea*
*Neisseria sicca*
*Neisseria subflava*
*Neisseria denitrificans*
*Peptostreptococcus species*
*Proteus mirabilis*
*Pseudomonas aeruginosa*
*Serratia marcescens*
*Staphylococcus aureus*

*Staphylococcus epidermidis*
*Streptococcus agalactiae*
*Streptococcus pneumoniae*
*Streptococcus pyogenes*
*Trichomonas vaginalis*
*Yersinia enterocolitica*

## Analytical sensitivity

The limit of detection (LoD) of the PRESTO CT/NG Test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was determined using quantified stock cultures (microscopically counted for CT, quantified DNA for NG). 20 replicates were tested at the concentrations of 0.02, 0.01 and 0.005 IFU/µl of CT and at 1.0, 0.5 and 0.25 G.Eq./µl of NG. The 20 CT replicates tested positive at 0.02 IFU/µl and 0.01 IFU/µl (Table 1). The 20 NG replicates tested positive at all three concentrations (Table 2). The LoD for the PRESTO CT/NG Test for CT is 0.1 IFU/test, for NG 2.5 G.Eq./test.

Table 1. LOD determination of the PRESTO CT/NG Test for CT.

| Concentration CT | $C_T$ (mean ± SD; n = 20) |
|---|---|
| 0.2 IFU/test | 34.46 ± 0.42 |
| 0.1 IFU/test | 36.16 ± 1.05 |
| 0.05 IFU/test | 18/20 Ct < 40 |

Table 2. LOD determination of the PRESTO CT/NG Test for NG.

| | $C_T$ (mean ± SD; n = 20) | |
|---|---|---|
| Concentration NG | NG strain 1 | NG strain 2 |
| 10 G. Eq./test | 33,5 ± 0.5 | 34,8 ± 0.3 |
| 5 G. Eq./test | 34.6 ± 0.4 | 35,7 ± 0.5 |
| 2.5 G. Eq./test | 35,7 ± 0.7 | 36,8 ± 0.9 |

For double infections: To determine the detection limit of CT in the presence of a high load of NG, 100 IFU, 10 IFU, 1 IFU and 0.1 IFU of CT per test were analysed in the presence of a high NG load (equimolar mix of 2 distinct NG strains; Ct < 22) with and without the selector for CT. Tests were carried out in triplicate. When CT was present in quantities < 100 IFU/test the high load of NG (Ct 21.7) decreased or masked the CT signal (Table 3). Addition of the CT selector restored the CT signal. The analytical sensitivity of the PRESTO CT/NG Test for *Chlamydia trachomatis* in the presence of high NG is 0.1 IFU/test.

Table 3. Sensitivity of the PRESTO CT/NG Test for CT in the presence of high load of NG ($C_T$ 21.7)

| | CT $C_T$ (mean ± SD; n = 3) | |
|---|---|---|
| | + IAC + NG | + IAC + NG + CT selector |
| 100 IFU/test | 24.56 ± 0.19 | 24.50 ± 0.05 |
| 10 IFU/test | 28.69 ± 0.46 | 27.75 ± 0.14 |
| 1 IFU/test | 0 out of 3 Ct's < 40 | 31.70 ± 0.28 |
| 0.1 IFU/test | 0 out of 3 Ct's < 40 | 36.04 ± 0.46 |

To determine the detection limit of NG in the presence of a high load of CT, 2500 G.Eq., 250 G.Eq., 25 G.Eq. and 2.5 G.Eq. of NG (equimolar mix of 2 distinct NG strains) per test were analysed in the presence of a high CT load ($C_T$ < 22) with and without the selector for NG. Tests were carried out in triplicate. When NG was present in quantities < 2500 G. Eq./test the high load of CT ($C_T$ 21.2) decreased or masked the NG signal (Table 4). Addition of the NG selector restored the NG signal. The analytical sensitivity of the PRESTO CT/NG Test for NG in the presence of high CT load is 2.5 G. Eq./test.

Table 4. Sensitivity of the PRESTO CT/NG Test for NG in the presence of high load of CT ($C_T$ 21,2).

| | NG $C_T$ (mean ± SD; n = 3) | |
|---|---|---|
| | + IAC + CT | + IAC + CT + NG selector |
| 2500 G.Eq/test | 26.1 ± 0.2 | 26.2 ± 0.2 |
| 250 G.Eq/test | 2 out of 3 Ct's < 40 | 29.0 ± 0.5 |
| 25 G.Eq/test | 1 out of 3 Ct's < 40 | 32.4 ± 0.1 |
| 2.5 G.Eq/test | 0 out of 3 Ct's < 40 | 36.9 ± 1.0 |

## Precision

The precision of the assay is shown in the table below (CV %):

| | Within run<br>n = 20 | Within day<br>3 time points<br>n = 3 | Day to day<br>10 days<br>n = 2 |
|---|---|---|---|
| **NG (G.Eq/Test)** | | | |
| 25 | 1.59 | 0.71 | 1.26 |
| 250 | 0.84 | 0.76 | 1.28 |
| 2500 | 0.97 | 0.43 | 1.77 |
| **CT (IFU/Test)** | | | |
| 1 | 0.82 | 0.82 | 1.07 |
| 10 | 0.75 | 0.80 | 0.57 |
| 100 | 0.26 | 0.58 | 0.39 |
| **Water + IAC** | | | |
| 83,3 CFU | 0.58 | 0.49 | 0.38 |

## Accuracy

The PRESTO CT/NG Test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was evaluated in a clinical study conducted at two different sites. A total of 4036 specimens were collected, i.e. swab (endovaginal, rectal, oropharyngeal) and urine specimens. The reference method is the Roche Amplicor CT/NG test.

Accuracy was determined by comparison with the Roche method. The two tables below give an overall overview.

Table below describes the results for the comparison of the Roche with the PRESTO kit for *Chlamydia trachomatis*. Total numbers are shown, those samples positive, negative and inhibited.

| **Presto** | | **Roche** | | |
|---|---|---|---|---|
| **CT** | **Total** | **Neg** | **Pos** | **Inhibited** |
| **Neg** | 3016 | 2965 | 17 | 34 |
| **Pos** | 319 | 16 | 300 | 3 |

Table below describes the results for the comparison of the Roche with the PRESTO kit for *Neisseria gonorrhoeae*. Total numbers are shown, those samples positive, negative, and inhibited / false positive.

| | | **Roche** | | |
|---|---|---|---|---|
| **Presto NG** | **Total** | **Neg** | **Pos** | **Inhibited / false Positive** |
| **Neg** | 3226 | 2877 | | 349 |
| **Pos** | 90 | 8 | 68 | 14 |

## Sensitivity, specificity, positive and negative predictive values

## (D.J. de Waaij et al. / Journal of Microbiological Methods 118 (2015) 70–74)

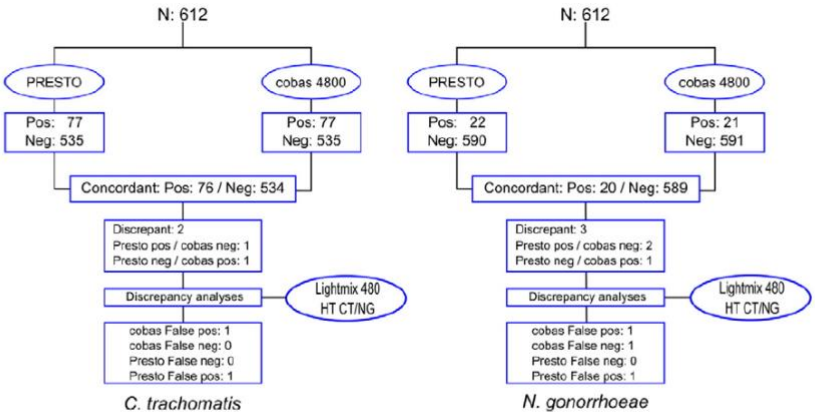



**Fig. 1.** Flow diagram of the results of vaginal CT and NG infections. The 612 samples, tested by the PRESTO and cobas® 4800 resulted in concordant and discrepant results. The Lightmix 480 HT CT/NG assay was used for discrepant samples and the gold standard was defined as two concurring results between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay.



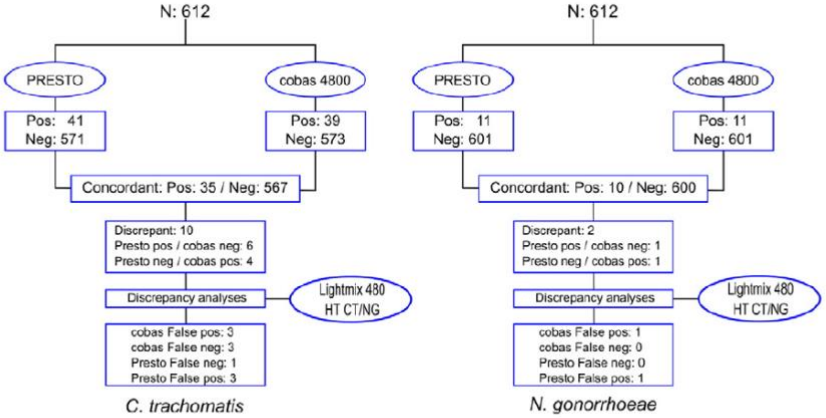



**Fig. 2.** Flow diagram of the results of anal CT and NG infections. The 612 samples, tested by the PRESTO and cobas® 4800 resulted in concordant and discrepant results. The Lightmix 480 HT CT/NG assay was used for discrepant samples and the gold standard was defined as two concurring results between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay.

**Table 1**
Sensitivity, specificity, positive predictive value, and negative predictive value for CT and NG for cobas® 4800 versus Presto.

| | | SENS% | 95% CI | SPEC% | 95% CI | PPV% | 95% CI | NPV% | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| Vaginal CT | Roche | 100.0 | 99.4–100.0 | 99.8 | 99.0–100.0 | 98.7 | 97.5–99.3 | 100.0 | 99.4–100.0 |
| | Presto | 100.0 | 99.4–100.0 | 99.8 | 99.0–100.0 | 98.7 | 97.5–99.3 | 100.0 | 99.4–100.0 |
| Vaginal NG | Roche | 95.2[#] | 93.3–96.7 | 99.8 | 99.1–100.0 | 95.2 | 93.2–96.7 | 99.8 | 99.1–100.0 |
| | Presto | 100.0[#] | 99.4–100.0 | 99.8 | 99.1–100.0 | 95.5 | 93.5–96.8 | 100.0 | 99.4–100.0 |
| Rectal CT | Roche | 92.3[*] | 89.9–94.2 | 99.5 | 98.5–99.6 | 92.3 | 89.9–94.2 | 99.5 | 98.5–99.8 |
| | Presto | 97.4[*] | 95.9–98.4 | 99.5 | 98.5–99.9 | 92.7 | 90.3–94.5 | 99.8 | 99.1–100.0 |
| Rectal NG | Roche | 100.0 | 99.4–100.0 | 99.8 | 99.1–100.0 | 90.9 | 88.4–92.9 | 100.0 | 99.4–100.0 |
| | Presto | 100.0 | 99.4–100.0 | 99.8 | 99.1–100.0 | 90.1 | 88.4–92.9 | 100.0 | 99.4–100.0 |

SENS, sensitivity; CI, confidence interval; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value.
The alloyed gold standard was a concurring result between the Presto and cobas® 4800 assays, or when these were discrepant, a concurring result between either Presto or cobas® 4800 assay and the Lightmix 480 HT CT/NG assay. Sensitivity, specificity, PPV, and NPV for both assays and both anatomical sites were calculated against the alloyed gold standard.
[#] 1 sample difference in sensitivity analyses.
[*] 2 sample difference in sensitivity analyses.

**Interfering Substances**

The presence of PCR inhibitors may cause false negative results. To check whether the IAC adequately monitors inhibition, one µl of the following substances was added to the PCR together with IAC at the concentration of 83.3 CFU/µl per test.

EDTA (0,5M)
ETOH 96%
DMSO 4 % (v/v)
HCl (1N)
Silica beads (1 µl)
Blood (1 µl)
Ureum (40 g/100ml)
Bilirubin (20 µM/L and 120 µM/L)
Vitamin C (56 µM/L)
Lysis buffer
Ciprofloxacin hydrochloride-1-$H_2O$ (trade name:Ciproxin) (2 mg/ml)
Vibramycin (20 mg/ml)
Metronidazol (5 mg/ml)

EDTA, HCl, Bilirubin (120 µM/L) and lysis buffer inhibited IAC-amplification completely, these substances were no further tested. In the presence of ethanol 96%, DMSO, silica beads, blood, ureum, bilirubin (20 µM/L), vitamin C, Ciprofloxacin hydrochloride-1- $H_2O$, Vibramycin and Metronidazol the IAC signal was still present. These substances were retested in the presence of CT and NG. The $C_T$ values for CT and NG in the presence of these non-inhibiting substances were all within 1 $C_T$ from the $C_T$ value of CT and NG in the presence of water. Thus, CT and NG amplification were not inhibited with these substances. The data show that IAC monitors inhibition well.

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## 11. Availability

For technical assistance please refer to the Catalogue Numbers:

**Kit # GM CG 160500**


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The sequences of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and the principle of the Isolation Amplification Control are licensed under patents WO2006014109; WO2008097082; JP2008508875.

For additional information, please visit www.goffinmt.com

## List of symbols as used in labelling

Contents (XXX µl)

Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices

For In Vitro Diagnostic Use Only.

## List of Abbreviations

| | |
|---|---|
| Ct | Cycle threshold |
| CT | *Chlamydia trachomatis* |
| IAC | Isolation Amplification Control |
| NG | *Neisseria gonorrhoeae* |
| PCR | Polymerase Chain Reaction |