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CT/NG DNA-Chip

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PelvoCheck® CT/NG

Your test kit for *Chlamydia trachomatis* screening and *Neisseria gonorrhoeae* infections

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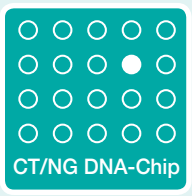
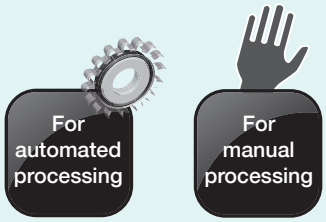


PelvoCheck® CT/NG

Instructions For Use



Diagnostic kit for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in human urine samples or vaginal and cervical swabs - automated processing.

For in-vitro diagnostic use
by professional laboratory personnel only

Revision: BQ-405-00 / June 2015

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Maybachstr. 2
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Germany
Made in Austria

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IVD

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Important information for the user

For the correct use of the Pelv**oCheck**® CT/NG, it is necessary for the user to carefully read and follow this instruction manual.

The manufacturer assumes no liability for any use of this system test which is not described in this document or for modifications by the user of the Test System.

INTENDED USE

Pelv**oCheck**® CT/NG is a diagnostic kit and intended to be used for the qualitative detection and differentiation of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) in human urine samples or vaginal and cervical swabs. Pelv**oCheck**® CT/NG may also be used for the qualitative detection of CT in human urine pools consisting of five pooled samples. Pelv**oCheck**® CT/NG must be used in combination with the listed equipment and by qualified personnel only.

The test kit is not intended for the analysis of any other sample material or the quantitative analysis of CT and NG load.

Pelv**oCheck**® CT/NG fulfils the requirements of the In Vitro Diagnostic Medical Device Directive (98/79/EC) and therefore displays the CE conformance mark. Any diagnostic result generated, using Pelv**oCheck**® CT/NG should be interpreted in conjunction with other clinical or laboratory findings.

The Pelv**oCheck**® CT/NG test kit (REF 504288) is recommended to be used together with the CheckExtractor™ for automated PCR setup. It is designed to process batches of 48 or 96 samples. It is NOT intended to process batches smaller than 48 samples.

GLOSSARY OF SYMBOLS

										
en	Batch code	Use by	Consult Instructions for Use	Catalogue Number	Manufacturer	In Vitro Diagnostic Medical Device	Temperature limitation	Contents sufficient for <n> tests	Danger	Store in the dark	Important Note
de	Chargenbezeichnung	Mindestens haltbar bis	Vor Gebrauch Anweisung lesen	Katalognummer	Hersteller	In-Vitro-Diagnostika Medizinprodukt	Temperaturbegrenzung	Inhalt ausreichend für <n> Tests	Gefahr	Im Dunkeln lagern	Wichtiger Hinweis
fr	N° de lot	Date limite de conservation jusqu'au	Lire les instructions avant utilisation	Numéro de référence	Fabricant	Produit médical de diagnostic in-vitro	Limite de température	Contenu suffisant pour <n> tests	Danger	À stocker à l'abri de la lumière	Note importante
es	Código de lote	A utilizar preferiblemente antes de	Antes de usar, lea las instrucciones	Número de catálogo	Fabricante	Producto medicinal de diagnóstica in vitro	Limitación de temperatura	Contenido suficiente para <n> ensayos	Peligro	Conservar en un lugar oscuro	Nota importante
it	Codice del lotto	Da utilizzare entro e non oltre	Leggere le istruzioni prima dell'uso	Numero catalogo	Produttore	Dispositivo medico-diagnostico in-vitro	Limitazione temperatura	Contenuto sufficiente per test <n>	Pericolo	Conservare al buio	Nota importante
pt	Código do lote	A utilizar preferívelmente antes de	Antes de usar, leia as instruções	Número de catálogo	Fabricante	Producto medicinal de diagnóstica in vitro	Limitação de temperatura	Conteúdo suficiente para <n> ensaios	Perigo	Conservar num local escuro	Aviso importante
nl	Lot nummer	Tenminste houdbaar tot	Gebruiksaanwijzing lezen	Catalogusnummer	Fabrikant	In vitro diagnostisch medisch product	Temperatuurbeperking	Voldoende inhoud voor <n> tests	Gevaar	Donker bewaren	Belangrijke opmerking
da	Lotnummer	Anvendes senest	Læs brugsanvisningen	Katalognummer	Producent	In vitro meskdicin doagnose-apparat	Temperaturbegrænsær	Indeholder nok til <n> test	Fare	Opbevares mørkt	Vigtig henvisning
sv	Lot nummer	Sista förbrukningsdag	Läs bruksanvisningen före användning	Katalognummer	Tillverkare	In vitro-medicinsk doagnostisk apparatur	Temperatur-begränsning	Innehållet räcker till <n> tester	Fara	Förvaras mörkt.	Viktigt meddelande
pl	Kod partii	Termin zydatności	Przed użyciem przeczytać instrukcję	Numer katalogowy	Producent	Diagnostyka in vitro Produktyw	Ograniczenie temperatury	Zawartość wystarcza na <n> testów	NIEBEZPIECZEŃSTWO	Przechowywać w ciemności	Ważne
no	batch nr.	holdbar til	Les bruksanvisning før bruk	katalognummer	produsent	in vitro-diagnostisk medisinsk utstyr	temperaturbegrensning	Innhold tilstrekkelig for <n> tester	Fare	Oppbevares mørkt	Viktig merknad
el	κωδικός παρτίδας	το λιγότερο διατηρείται	πριν την χρήση διαβάστε τις οδηγίες	Αριθμός Καταλόγου	Παραγωγός	In vitro διαγνωστικά ιατρικά προϊόντα	περιορισμός θερμοκραοίας	Περιεχόμενο αροκετό για <n> τεοτ	ΚΊΝΔΥΝΟΣ	Αποθηκεύεται στα σκοτεινά	Σημαντική υπόδειξη
tr	Parti kodu	Son kullanma tarihi:	Kullanmadan önce talimatı okuyun	Katalog numarası	Üretici firma	In vitro diagnostik tıbbi tanı ürünü	Sıcaklık sınırlaması	İçeriği <n> test için yeterlidir	Tehlikeli	Karanlık yerde saklayınız	Önemli Not

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1. KIT CONTENT

PelvoCheck® CT/NG test kit[1]	Content	Quantity
PelvoCheck® CT/NG PCR-MasterMix	6 x PelvoCheck® CT/NG PCR MasterMix[2]	6 x 1,200 µL
MasterMix preparation	6 x vials for MasterMix preparation (ready to use)	6 x empty
PelvoCheck® CT/NG Slidebox, 4 x 12 Arrays	6 x PelvoCheck® CT/NG Slidebox with 4 PelvoCheck® CT/NG chips[3]	6
PelvoCheck® CT/NG Hyb.-Buffer	12 x PelvoCheck® CT/NG Hybridisation Buffer (ready to use)	12 x 1,200 µL
PelvoCheck® CT/NG BUF A conc.	1 x PelvoCheck® CT/NG Buffer A concentrate	1 x 450 mL
PelvoCheck® CT/NG BUF B conc.	1 x PelvoCheck® CT/NG Buffer B concentrate	1 x 55 mL
	Download Instructions for Instructions for Use	1

The main packaging contains one bottle each of Buffer A and B concentrate and six small cardboard boxes. Each of the small boxes contains further materials for 48 analyses and therefore: one vial of PCR MasterMix, one vial for MasterMix preparation, two vials of Hybridisation Buffer and one Slidebox with four PelvoCheck® CT/NG chips. This packaging supports the fact that this kit is not intended to be used to process batches smaller than 48 samples.

[1] One PelvoCheck® CT/NG test kit is sufficient for the analysis of 288 reactions.
[2] Contains all components required for PCR except for the DNA Polymerase and the Uracil-N-Glycosylase.
[3] One PelvoCheck® CT/NG chip contains 12 PelvoCheck® CT/NG microarrays.

2. CONSUMABLES, EQUIPMENT AND HARDWARE REQUIRED

PelvoCheck® CT/NG is recommended to be used in combination with the listed consumables, equipment and hardware and by professional personnel only.

Consumables	Greiner Bio-One Cat. No.	Quantity
PelvoCheck® CT/NG test kit	504 288	Test kit for 288 reactions
PelvoCheck® Collection Kit SAFE PelvoCheck® Collection Kit STRAW PelvoCheck® Swab Collection Kit	453 100 453 101 453 103	50 samples 70 samples 60 samples
oCheck® DNA Extraction Kit / Single Column Preparation	515 040	50 preparations
oCheck® DNA Extraction Kit - CheckExtractor™[5]	517 070	288 preparations
Sterile, DNase-free micropipette filter tips[4] 10 µL low retention filter tips 100 µL low retention filter tips 1250 µL low retention filter tips	 771 265 737 261 750 265	 96/960 96/960 96/768
Pipette tips for CheckExtractor™[5] 300 µL Pipette tips 1000 µL Pipette tips	 865 807 866 806	 5760 3890
DNase-free reaction tubes Sample tubes Caps for the sample tubes Reagent troughs 60 mL Reaction tube 1.5 mL 96 Well Polypropylene Microplates, with half skirt, suitable for ABI	 481 478 481 479 865 808 616 201 652 290-CEX	 1000 1000 28 500/4000 40
MASTERBLOCK®[5] MICROLAB™[5] Detergent & Disinfectant MICROLAB™[5] Disinfectant Spray Waste bag[5]	780 270 865 809 865 810 865 815	50 1 1 25
Sealing Pearce Seal Silver Seal	 865 804 676 090	 100 100
Plastic pipettes for pipettor Pipette 10 ml Pipette 25 ml Pipette 50 ml	 607 180 or 607 160 760 180 or 760 160 768 180 or 768 160	 1/200 1/200 1/200

[4] Some of the mentioned tip sizes are optional depending on the micropipettes available.
[5] Only required for automated processing using CheckExtractor™.

Equipment	Greiner Bio-One Cat. No.	Quantity
CheckExtractor™	863 080	1
Plate centrifuge	865 805	1
CheckScanner™	862 070	1
CheckReport™Software Basic	862 080	1
CheckReport™Software Pelv**oCheck**® CT/NG plugin	862 086	1
PC system including CheckReport™Software and Pelv**oCheck**® CT/NG plugin	862 910	1
Starter Package CheckScanner™ (CheckScanner™, PC system, CheckReport™Software, Pelv**oCheck**® CT/NG plugin)	862 170	1
oCheck® Hybridisation Chamber with slideholder	447 070	1
Magnetic handle for slide holder	447 001	1
oCheck® Washbox[6]	447 020	1

Enzymes required

- Polymerase: HotStarTaq® DNA Polymerase 5 U/µL (mandatory: Qiagen; 203203, 203205, 203207, 203209)
- Uracil-N-Glycosylase: Uracil-DNA Glycosylase 1 U/µL (mandatory: Thermo Scientific; #EN0362)
- Proteinase K[7]

Additional consumables required

- PCR-grade water
- Purified water
- Single-use gloves

Additional equipment required

- PCR thermal cycler:
  GeneAmp® PCR system 9700 (Applied Biosystems) or Veriti™ 96-Well Thermal Cycler (Applied Biosystems)
- Water bath (50 °C)
- Micropipettes (different ranges from 1 - 1,000 µL)
- 8-Channel multipipette (range: 5 - 50 µL)
- Pipettor for glass and plastic pipettes
- Vortex shaker
- Racks for different reaction tubes
- Timer
- Waste container

Additional hardware required

- Computer (for system requirements see Instructions For Use of the CheckScanner™ and the CheckReport™Software)

[6] For the Pelv**oCheck**® CT/NG washing procedure two oCheck® Washboxes are required
[7] Only required for urine pooling): 30 mg and 1.8 mL Proteinase K Buffer (for pooling only) (Greiner Bio-One REF 515 041)

3. SHIPMENT AND STORAGE

The shipment of the Pelv**oCheck**® CT/NG test kit takes place at ambient temperature conditions. Nevertheless, the kit has to be stored immediately upon receipt at 4 to 8 °C and should be protected from light. Keep the slidebox with the Pelv**oCheck**® CT/NG chips always in the closed zipper bag with desiccant. All components must be stored in the original kit packaging to avoid mixed batches or mixed expiration dates.

Stored correctly, the Pelv**oCheck**® CT/NG test kit and its components can be used until the indicated expiration date. Furthermore, under these conditions the shelf life does not deviate from the expiration date after the first opening of the kit and its components.

Product	Storage
Pelv**oCheck**® CT/NG	4 to 8 °C, protected from light

4. SAFETY INSTRUCTIONS

The Pelv**oCheck**® CT/NG test kit is for laboratory use only, not for drug, household, or other purposes. The product is intended for professional users only, such as technicians or physicians trained in molecular biology techniques.

! Always wear a suitable lab coat, disposable gloves and protective goggles and follow the safety instructions given in this section and section 7.4.

Regulatory Information:
The following components of the Pelv**oCheck**® CT/NG test kit contain harmful or hazardous contents:

Kit Component Quantity Hazardous content	Classification according to Regulation (EC) No. 1272/2008	GHS picto-gram and signal word	Hazard and precautionary statements	
Pelv**oCheck**® CT/NG Hybridisation Buffer, guanidine thiocyanate solution, 25-50 %, CAS No. 593-84-0	acute toxicity, oral (category 4), acute toxicity, inhalation (category 4), chronic aquatic toxicity (category 3) skin corrosive (category 1c)	 DANGER	H302 H332 H314 H412 P273 P280 P305+ P351+P338 EUH032	Harmful if swallowed. Harmful if inhaled. Causes severe skin burns and eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/physician. Supplemental hazard information (EU): Contact with acids liberates very toxic gas.
Pelv**oCheck**® CT/NG Buffer B, sodium dodecyl sulfate solution, 10-20 %, CAS No. 151-21-3	skin irritation (category 2), serious eye damage (category 1)	 DANGER	H315 H318 P280 P305+ P351+P338	Causes skin irritation. Causes serious eye damage. Wear protective gloves/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

The current version of the Safety Data Sheet for this product can be downloaded from the Greiner Bio-One website: **www.gbo.com/en_INT/know-how-services/download-center.html**

5. WASTE DISPOSAL

After washing and drying of the Pelv**oCheck**® CT/NG chip, the washing solutions I and II can be discarded without any special precautions. Dispose the used Pelv**oCheck**® CT/NG chip, unused kit components as well as unused hybridisation mix with the laboratory chemical waste.

Follow all national, state, and local regulations regarding disposal.

6. INTRODUCTION

6.1 Background Information

Pelvic inflammatory disease (PID) is the most common and severe complication of some sexually transmitted infections (STI) and refers to inflammation of the uterus, the fallopian tubes, and/or ovaries progressing to scar formation with adhesions to nearby tissues. This may damage the tissues with serious consequences, such as chronic pelvic pain, abscess formation, ectopic pregnancy and infertility. The true incidence and prevalence of PID is difficult to estimate, because subclinical cases are easily missed and PID is not a notifiable disease. General practice data from England and Wales suggest a PID prevalence of 1.7 %[8] and the CDC has estimated that each year more than 750.000 women in U.S. experience an episode of acute PID[9].

The majority of PID cases are caused by the sexually transmitted pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. CT and NG are non-motile and gram-negative bacteria. The CT species are obligat intracellular and comprise of 15 serovars (A, B, Ba, C, D, E, F, G, H, I, J, K, L1, L2 and L3) that can cause disease in humans[10]. The serovars D through K are the major cause of genital chlamydial infections in men and women[11]. Pelv**oCheck**® CT/NG detection is based on the amplification of a highly conserved region of the 16S rRNA gen and therefore, the assay can detect all CT serovars and the swedish variant containing a deletion in the cryptic plasmid of CT.

CT/NG move upwards from a woman's vagina or cervix into her reproductive organs. In pregnant women untreated chlamydial infections can also endanger the new-born leading to conjunctivitis, atypical pneumonia and infant blindness. Chlamydia is the most frequently reported STI in Europe[14]. Population-based studies found a prevalence of *Chlamydia trachomatis* infections between 1.4 % and 8 % depending on the age group[12,13]. Three quarter of all Chlamydia cases are reported in young people of 15-24 years[11].

Although STIs and PID may be cured, their effects can be permanent. This makes early identification essential to prevent long-term effects and further spread of the pathogen. In this context, partner notification and treatment is an important measure to prevent re-infection and STI propagation.

In the recent past, several national health authorities have started Chlamydia screening programs for expanded Chlamydia testing in adolescent, who have the highest risk for infection and PID development[15]. It is recommended that all sexually active adolescent women and men (men not in Germany) are screened for Chlamydia at least once a year.

---

[8] Simms, I.; Stephenson, J.M. (2000) Pelvic inflammatory disease epidemiology: what do we know and what do we need to know?, Sexually Transmitted Infections 76:80-87

[9] Center for Disease Control and Prevention: http://www.cdc.gov/std/PID/STDFact-PID.htm, Page last reviewed: March 25, 2011, Page last updated: September 28, 2011

[10] Schachter, J. 1985. Chlamydiae (Psittacosis-Lymphogranulom a Venereum-Trachoma group), p. 856-862. In E. H. Lennette, et al. (ed.), Manual of Clinical Microbiology, 4 th ed. American Society for Microbiology, Washington, D.C.

[11] Yuan, Y., Y-X. Zhang, N. G. Watkins, and H. D. Caldwell. 1989. Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 Chlamydia

[12] Adams, E.J. et al. (2004) *Chlamydia trachomatis* in the United Kingdom: a systematic review and analysis of prevalence studies, Sexually Transmitted Infections 80:354-362

[13] Van Bergen, J. et al. (2005) Prevalence of urogenital *Chlamydia trachomatis* increases significantly with level of urbanisation and suggests targeted screening approaches: results from the first national population based study in the Netherlands, Sexually Transmitted Infections 81:17-23

[14] European Center for Disease Prevention and Control (ecdc), Surveillance Report – Annual epidemiological report 2011, Stockholm, ISBN: 978-92-9193-321-1

[15] European Center for Disease Prevention and Control (ecdc), Chlamydia control in Europe – June 2009, Stockholm, ISBN 978-92-9193-165-1

6.2 Assay principle

Pelv**oCheck**® CT/NG is a microarray-based test kit for the DNA-based detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). The assay procedure, outlined in Figure 1, is based on the detection of a 16S rRNA gene fragment specific for these pathogens.

Prior to the Pelv**oCheck**® CT/NG analysis, DNA must be extracted from urine or genital swab samples. Sample collection and DNA extraction are not part of the Pelv**oCheck**® CT/NG test kit. Dedicated products for sample collection (Pelv**oCheck**® Collection Kit SAFE and STRAW, and Pelv**oCheck**® Swab Collection Kit) and DNA extraction (oCheck® DNA Extraction Kit or oCheck® DNA Extraction Kit - CheckExtractor™) are also available from Greiner Bio-One and must be separately purchased (see ordering information in Chapter 2).

After the extraction of bacterial and human genomic DNA from urine or genital swab samples, a 200-300 bp fragment of the 16S rRNA gene is amplified by polymerase chain reaction (PCR)[16] in the presence of a set of CT- and NG-specific primers. In the same reaction, a fragment of the human single-copy gene ADAT1 (human tRNA-specific adenosine deaminase1) is amplified to monitor the presence of human sample material in the urine or genital swab samples (sample control) and an internal control-template present in the Pelv**oCheck**® CT/NG PCR MasterMix is amplified to monitor the performance of the PCR (PCR control). During PCR, the amplified DNA is fluorescently labelled. In addition, the Pelv**oCheck**® CT/NG PCR MasterMix contains dUTP. Thus, potential carry-over contamination from previous PCR reactions can be eliminated through the use of Uracil-N-Glycosylase (UNG) treatment (see Chapters 8.2.6 and 8.3.5).

The PCR products are then hybridised to pathogen-specific and control-DNA probes attached to the Pelv**oCheck**® CT/NG chip surface. Every chip contains 12 DNA-microarrays, allowing the simultaneous analysis of up to 12 samples (urine or swab samples and negative control samples). Unbound DNA is removed in the subsequent washing steps. The hybridisation efficiency is monitored (hybridisation control). Finally, the Pelv**oCheck**® CT/NG chip is automatically scanned, analysed and evaluated using the CheckScanner™ and CheckReport™Software, respectively (see ordering information in Chapter 2).
The CheckScanner™ is a two colour laser scanner (excitation wavelengths of 532 nm and 635 nm), which enables the detection of the fluorescence signal generated by the presence of specific amplification products as well as the controls (see Chapter 6.3.2). The CheckReport™Software allows the visualisation, analysis and evaluation of the results and automatically shows the corresponding values of both the detected pathogens and the controls in a detailed and a summary report.
The report clearly indicates the presence or absence of CT and/or NG and the comprehensive on-chip controls render the analysis highly reliable.

[16] The PCR process is covered by U.S. patents owned by Hoffmann-La Roche Inc. Use of the PCR process requires a license. Nothing in this publication should be construed as an authorization or implicit license to PCR under patents held by Hoffmann – La Roche Inc.

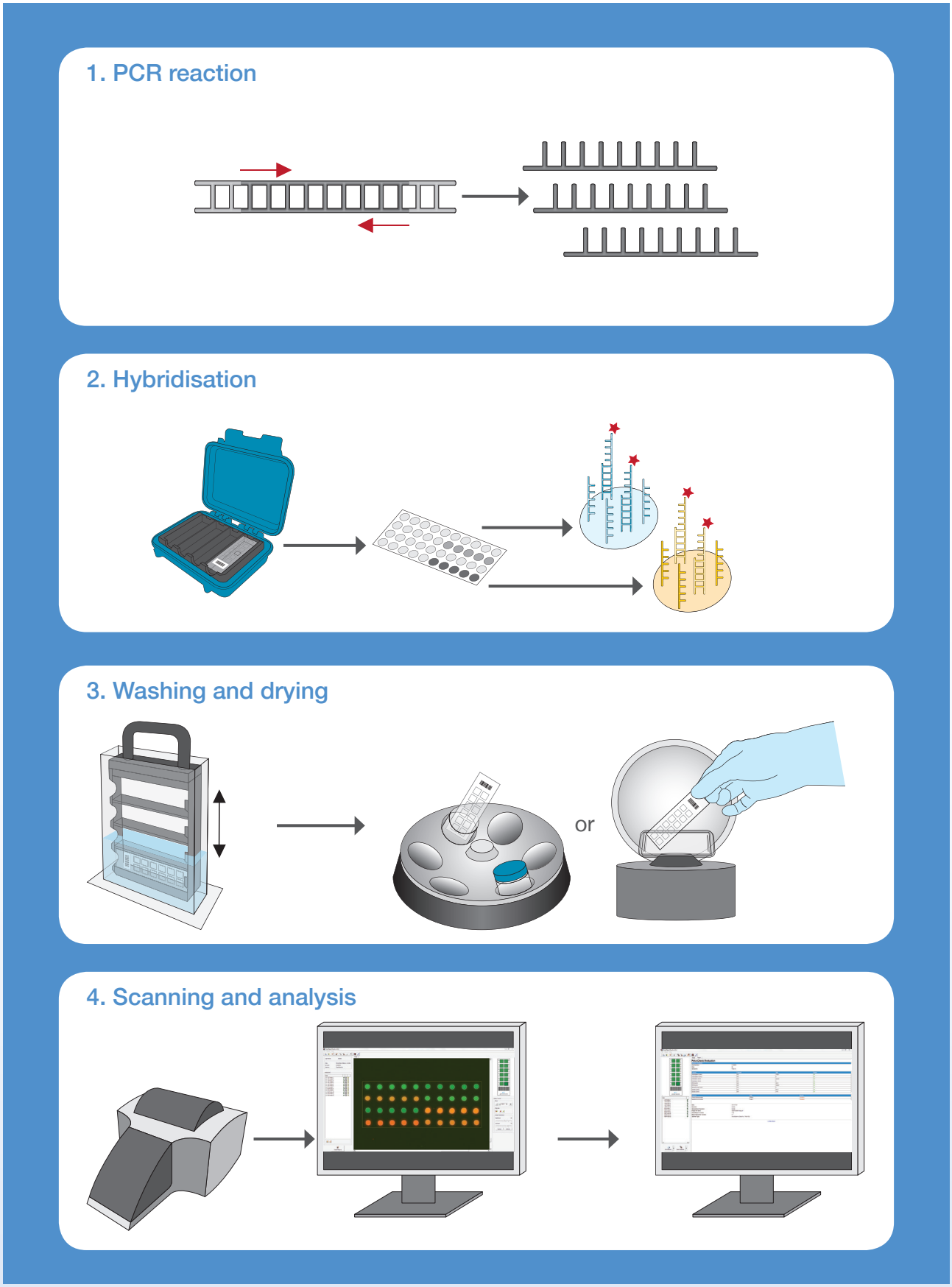


Figure 1: PelvoCheck® CT/NG assay procedure

1. PCR reaction: After DNA extraction, a 16S rRNA fragment of about 200-300 nucleotides and fragments of two control targets are PCR-amplified in the presence of specific primers. During PCR the fluorescence label is introduced.
2. Hybridisation: The amplification products are then hybridised to complementary DNA probes present in five replicas on each array.
3. Washing and drying: During subsequent washing steps, unbound amplification products are washed away.
4. Scanning and analysis: The PelvoCheck® CT/NG chip is scanned, analysed and evaluated using the CheckScanner™ and CheckReport™Software. A report is created that clearly indicates the presence or absence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*.

6.3 Design of the PelvoCheck® CT/NG chip

6.3.1 PelvoCheck® CT/NG chip layout

Each Pelv**oCheck**® CT/NG chip contains 12 microarrays designated as well A1 - B6. Each Pelv**oCheck**® CT/NG microarray comprises of 7 different probes and is bordered by an elevated rim. Each probe is spotted in five replicates. Three type-specific probes enable the detection of the CT and NG (NG_1 and NG_2) using the red channel (excitation wavelength of 635 nm). Four process controls and the printing control of all spots monitor the assay procedure in the green and the red channel (excitation wavelength of 532 and 635 nm). The Pelv**oCheck**® CT/NG microarray layout is illustrated in Figure 2 and the on-chip controls are further explained in Chapter 6.3.2.

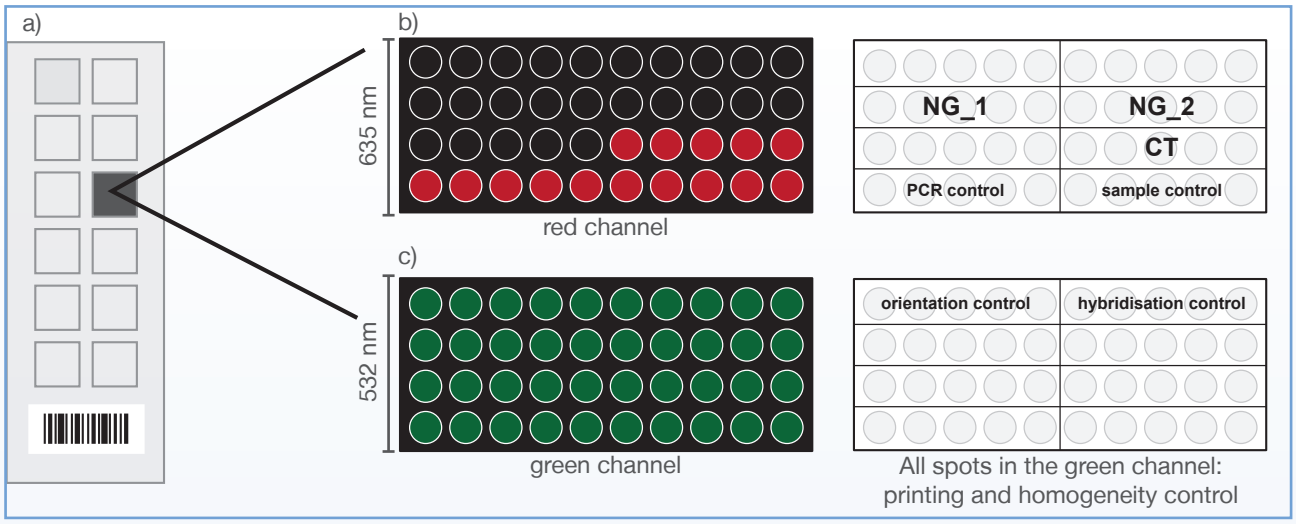


Figure 2: Design of the PelvoCheck® CT/NG chip
a) Schematic drawing of the Pelv**oCheck**® CT/NG chip with 12 independent compartments containing each one microarray. b) and c) Microarray images displayed by the CheckReport™Software for the two different excitation wavelengths used for scanning (b) red channel: 635 nm; c) green channel: 532 nm) and schematic drawings of the Pelv**oCheck**® CT/NG chip layout. Pathogen-specific probes and on-chip controls are indicated.

6.3.2 On-chip controls

The design of the Pelv**oCheck**® CT/NG DNA-chip incorporates comprehensive on-chip controls. Several control systems monitor all critical steps of both the assay and chip processing, including sample quality and DNA extraction efficiency (sample control), performance of the PCR reaction (PCR control), the efficiency of the hybridisation (hybridisation control), as well as spot homogeneity and printing quality (orientation control and printing control). In addition to the presence or absence of CT and/or NG the CheckReport™Software automatically shows both the corresponding values of the controls and the detected pathogens in a detailed report. For read-out of the different controls, both excitation wavelengths of the CheckScanner™ are used. For the control of sampling and DNA extraction efficiency as well as PCR performance (sample and PCR control), the red channel is used (excitation wavelength of 635 nm), while the quality of the hybridisation and the chip (hybridisation, orientation and printing control) is assessed in the green channel (excitation wavelength of 532 nm).

Red channel (Excitation wavelength 635 nm)

Sample control
Pelv**oCheck**® CT/NG monitors the quality of the sample, its sampling and/or the DNA extraction by amplifying a fragment of the human single-copy gene ADAT1 (human tRNA-specific adenosine deaminase1). If human DNA is present in an adequate amount in the DNA extract from the sample, a fluorescence signal on the sample control spots is generated.
If no or insufficient ADAT1 amplification occurs, the CheckReport™Software will indicate the sample control as “failed”. This has two different consequences, depending on the signal from the pathogen-specific spots. If the sample is positive for CT and/or NG, the analysis remains valid. In this case, the failed sample control is a direct result of competition between human DNA and an excess of bacterial DNA during PCR. If, on the other hand, the sample is negative for CT and NG, the CheckReport™Software will indicate the analysis as “failed” due to poor sample collection (insufficient amount of cells in the sample) and/or inefficient DNA extraction. In this case, it is recommended to repeat the analysis beginning with the DNA extraction or, if necessary, to take a new sample (see Chapter 9 “Troubleshooting”).

PCR control
Pelv**oCheck**® CT/NG also monitors the performance of the PCR. Amplification of an internal control template present in the Pelv**oCheck**® CT/NG PCR MasterMix generates a signal on the PCR control spots on the Pelv**oCheck**® CT/NG chip. The quality of the amplification reaction is also automatically assessed by the CheckReport™Software. If PCR performance is below a predefined threshold, the CheckReport™Software will indicate the PCR control as “failed” and the analysis must be repeated (see Chapter 9 “Troubleshooting”).

If the amount of bacterial DNA in the sample is very high, the fluorescence signal of the PCR control spots may be low or even absent due to competition during the PCR reaction. In this case, the fluorescence signal for at least one pathogen-specific probe must exceed a predefined threshold in order for the test to be considered valid.

Green channel (Excitation wavelength 532 nm)

Hybridisation control
Pelv**oCheck**® CT/NG monitors the efficiency of the hybridisation through use of a fluorescence labelled probe within the Pelv**oCheck**® CT/NG Hybridisation Buffer, which hybridises to specific DNA sequences on the Pelv**oCheck**® CT/NG chip. Adequate hybridisation efficiency results in fluorescence signals on each array spot. The results of five hybridisation control spots on the Pelv**oCheck**® CT/NG chip are assessed by the CheckReport™Software to evaluate the performance of the hybridisation reaction.

Orientation and printing control

The orientation control spots of the Pelv**oCheck**® CT/NG chip generate fluorescence signals irrespective of the efficiency of the hybridisation process. These spots are used by the CheckReport™Software as guidance points for a correct spot finding, which is a prerequisite for the correct analysis of the signals. In addition, the quality of the printing process is monitored by the presence of a green fluorescence signal at each chip spot (printing control).

7. INSTRUCTIONS FOR THE PELVOCHECK® CT/NG WORKFLOW

7.1 General instructions

When implementing currently used state-of-the-art techniques in molecular biology into a laboratory, the following instructions must be considered to ensure both maximum safety for laboratory staff and high quality results.
Execution of molecular biology techniques such as DNA extraction, amplification and detection of the amplification products require appropriately qualified personnel. In addition, a clean and well-structured workflow is required to prevent erroneous results, such as those occurring due to DNA degradation or contamination by amplification products. To ensure this, it is necessary to separate the areas of extraction, amplification and detection as described in Chapter 7.2 and 7.3. Each area should be equipped with separate equipment, consumables, lab coats and gloves. Never transfer lab coats, gloves or equipment from one distinct area to another.

7.2 Room separation for manual processing

It is absolutely necessary to separate the areas of DNA extraction, amplification, and hybridisation. An amplification product should never be introduced in the area that is intended for extraction and sample material must not be introduced in the area for setting up the amplification PCR MasterMix.

Figure 3 shows an example of how a laboratory may be separated into three distinct sections. One is used only for DNA extraction, another is for the set-up and running of PCR reactions and the last is for hybridisation and analysis. Each room is used exclusively for the application or technique indicated to prevent sample contamination. The use of colour coding could be advantageous to avoid the accidental exchange of equipment and consumables between areas.

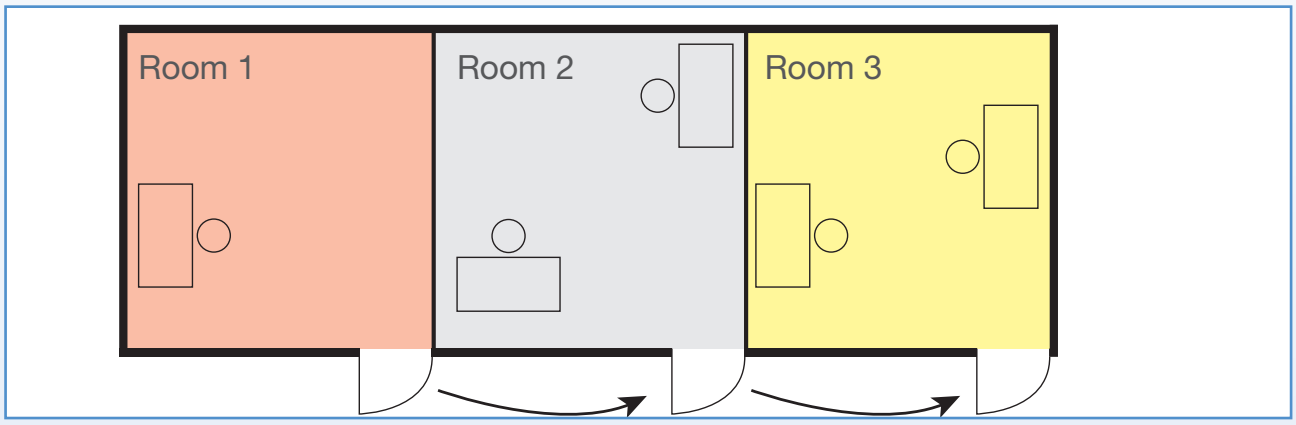


Figure 3: Room separation for manual processing

Room 1: The entire DNA extraction procedure must be performed in this room.
Room 2: Within this room, the final PCR MasterMix for the PCR is setup and aliquoted (optimally under a PCR hood). The addition of the DNA samples extracted in room 1 must be carried out in a separate space within room 2.
Room 3: Within the third laboratory room the hybridisation reaction, washing steps and chip drying take place. Additionally, the CheckScanner™ in combination with the CheckReport™Software is used for the final analysis of the PelvoCheck® CT/NG assay.

! Neither equipment nor consumables should be interchanged between the different laboratory rooms and spaces. Hence, duplications in equipment and consumables are a necessity and should be taken into account when equipping the laboratory.

7.3 Room separation for automated processing

It is absolutely necessary to separate the areas of DNA extraction, amplification, and hybridisation. An amplification product should never be introduced in the area that is intended for extraction and sample material must not be introduced in the area for setting up the amplification PCR MasterMix.

Figure 4 shows an example of how a laboratory may be separated into three distinct sections. One is designated for DNA extraction with the CheckExtractor™ and the processing of the PCR plate. The setup of the PCR MasterMix should be done in a clean bench in room 2 and the hybridisation and analysis in room 3. Each room is used exclusively for the application or technique indicated to prevent sample contamination. The use of colour coding could be advantageous to avoid the accidental exchange of equipment and consumables between areas.

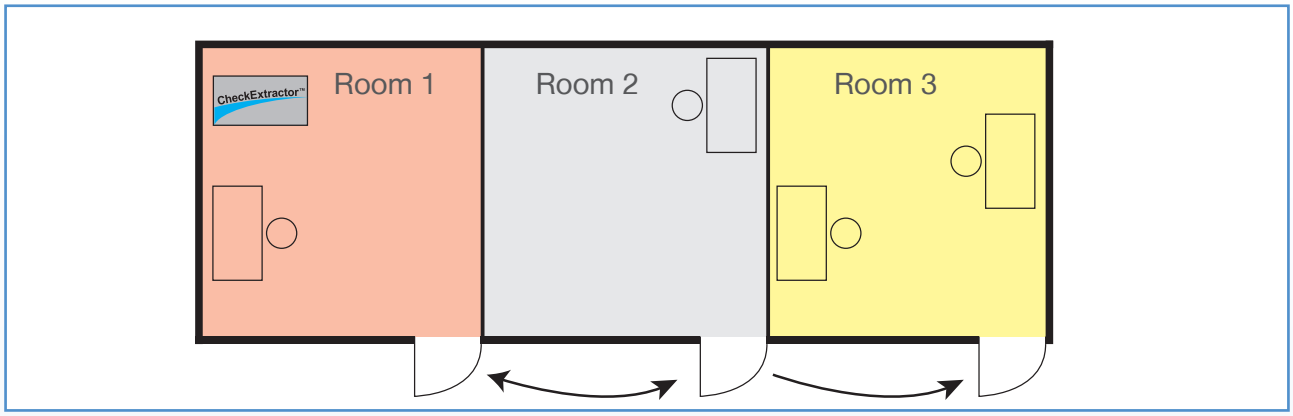


Figure 4: Room separation for automated processing

Room 1: The entire DNA extraction procedure must be performed in this room.
Room 2: Within this room, the final PCR MasterMix for the PCR is set up (optimally under a PCR hood). The final PCR MasterMix is transferred back to the CheckExtractor™ in room 1 for performing the PCR setup.
Room 3: Within the third laboratory room the hybridisation reaction, washing steps and chip drying take place. Additionally, the CheckScanner™ in combination with the CheckReport™Software is used for the final analysis of the Pelv**oCheck**® CT/NG assay.

!

Neither equipment nor consumables should be interchanged between the different laboratory rooms and spaces. Hence, duplications in equipment and consumables are a necessity and should be taken into account when equipping the laboratory.

7.4 Warnings and precautions

7.4.1 Contamination prevention

- **Lab coats** must be worn throughout the procedures and different sets of lab coats are needed for each laboratory room.
- **Lab cleanness:** The working place must be decontaminated with DNA-AWAY® or any other appropriate cleaning solution before and after work.
- **Gloves** must be worn during each step of the analysis and must be changed frequently, especially during DNA extraction.
- **Sample tubes:** Never touch the inside of a reaction tube cap. To avoid cross-contamination, open only one tube at a time.
- **Pipetting:** Appropriate micropipette filter tips with aerosol barriers must be used (sterile, free of DNase, RNase and human DNA). Pipette tips should always be changed between liquid transfers.

7.4.2 General precautions

- Upon arrival, check the kit components for damage. If one of the components is damaged (e.g. buffer bottles), contact your local Greiner Bio-One distributor. Do not use damaged kit components, as their use may lead to poor kit performance.
- Do not use the kit after the expiry date.
- Do not mix reagents from different batches.
- Do not use expired reagents.
- Use only reagents/equipment provided with the kit and those recommended by the manufacturer.
- Regular calibration/maintenance should be performed for micropipettes, waterbath, heating block and thermal cycler.
- Unused reagents and waste material must be disposed of in accordance with federal and state guidelines.
- Pipetting of small amounts of liquid in the microliter range is a challenge. Therefore take care to pipette as accurately as possible.
- To avoid microbial contamination of the reagents, take care when removing aliquots from reagent tubes.
- Unused reagents and waste material must be disposed in accordance with federal and state guidelines.
- All centrifugation steps should be carried out at room temperature (18 to 25 °C).

7.4.3 Working safely

- Always wear a suitable lab coat, disposable gloves and protective goggles!
- This kit is for in vitro diagnostic use only and should be exclusively used by personnel trained in in vitro diagnostic laboratory practice.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Take care whilst handling biological samples containing potential human infectious material. To minimise the risk of infection from potentially infectious material, it is recommended to work under laminar air-flow conditions. Handle and dispose of all biological samples as if they were capable of transmitting infectious agents.
- Avoid direct contact with the biological samples as well as splashing or spraying. Always wear lab coat, gloves and goggles while working with human samples.
- Wash hands carefully after handling samples and reagents.

7.4.4 Instruction for handling chips

- DNA chips should be used in a dust-free environment. The deposition of dust and other particles on the chip surface must be prevented.
- Do not touch the hybridisation zone on the chip surface.
- Only the labelled side of the chip is intended for hybridisation.
- Do not use any marker pens for the identification of DNA chips, as they lead to unspecific fluorescence on the chip.
- DNA chips are for single use only. Hybridised chips cannot be reused.
- Store unused chips in the original box inside the delivered zipper bag containing the desiccant.

The current version of the Material Safety Data Sheet for this product can be downloaded from the Greiner Bio-One website: **www.gbo.com/en_INT/know-how-services/download-center.html**

8. PELVOCHECK® CT/NG PROCEDURE

The following chapter describes in detail the different working steps which finally lead to the production of a detailed report, clearly indicating the presence or absence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* in each analysed sample. Please be aware that the following sections are divided into manual and automated processing.

Figure 5 (see page 23) shows an overview of the necessary different working steps. It also indicates the corresponding subchapter describing the specific assay step. The working steps must be performed in the order outlined in this chapter. Each specific hands-on step is indicated by a blue arrow ➠.

! Sample collection, DNA extraction and analysis with the CheckReport™Software are not part of the Pelv**oCheck**® CT/NG test kit. Therefore, the description of these working steps is abbreviated within this chapter. For more detailed information, please refer to the corresponding Instructions For Use, e.g. from the oCheck® DNA Extraction Kit - CheckExtractor™, CheckReport™Software, Pelv**oCheck**® Collection Kits (SAFE and STRAW), Pelv**oCheck**® Swab Collection Kit and oCheck® DNA Extraction kit.

! Please read the Instructions For Use carefully. In case you are already using the Papill**oCheck**® test kit for HPV testing, please take care to notice the differences between the protocols.

8.1 Sample collection and DNA extraction

8.1.1 Sample collection

Specimen collection is not part of the Pelv**oCheck**® CT/NG test kit. Two dedicated collection kits for urine specimens (Pelv**oCheck**® Collection Kit SAFE or STRAW) as well as a collection kit for vaginal and cervical swabs (Pelv**oCheck**® Swab Collection Kit) are available from Greiner Bio-One (see ordering information in Chapter 2). Please carefully follow the detailed instructions described in the Instructions For Use of the Pelv**oCheck**® Collection Kit and Pelv**oCheck**® Swab Collection Kit.

For Pelv**oCheck**® CT/NG analysis from urine samples, the collection of first-void urine, corresponding to the first 20-30 mL of the urine stream, is a prerequisite for a sensitive detection of the pathogens. This is especially important for the detection of CT, which is an obligate intracellular organism, while NG is found predominately cell-associated. Normally, the first-void urine is abound in cells and only sampling of this fraction enables a sensitive Pelv**oCheck**® CT/NG analysis according to the indicated performance characteristics.

! Urine samples cannot be concentrated by centrifugation, because the collection medium of the Pelv**oCheck**® Collection Kits SAFE and STRAW lyses the cells.

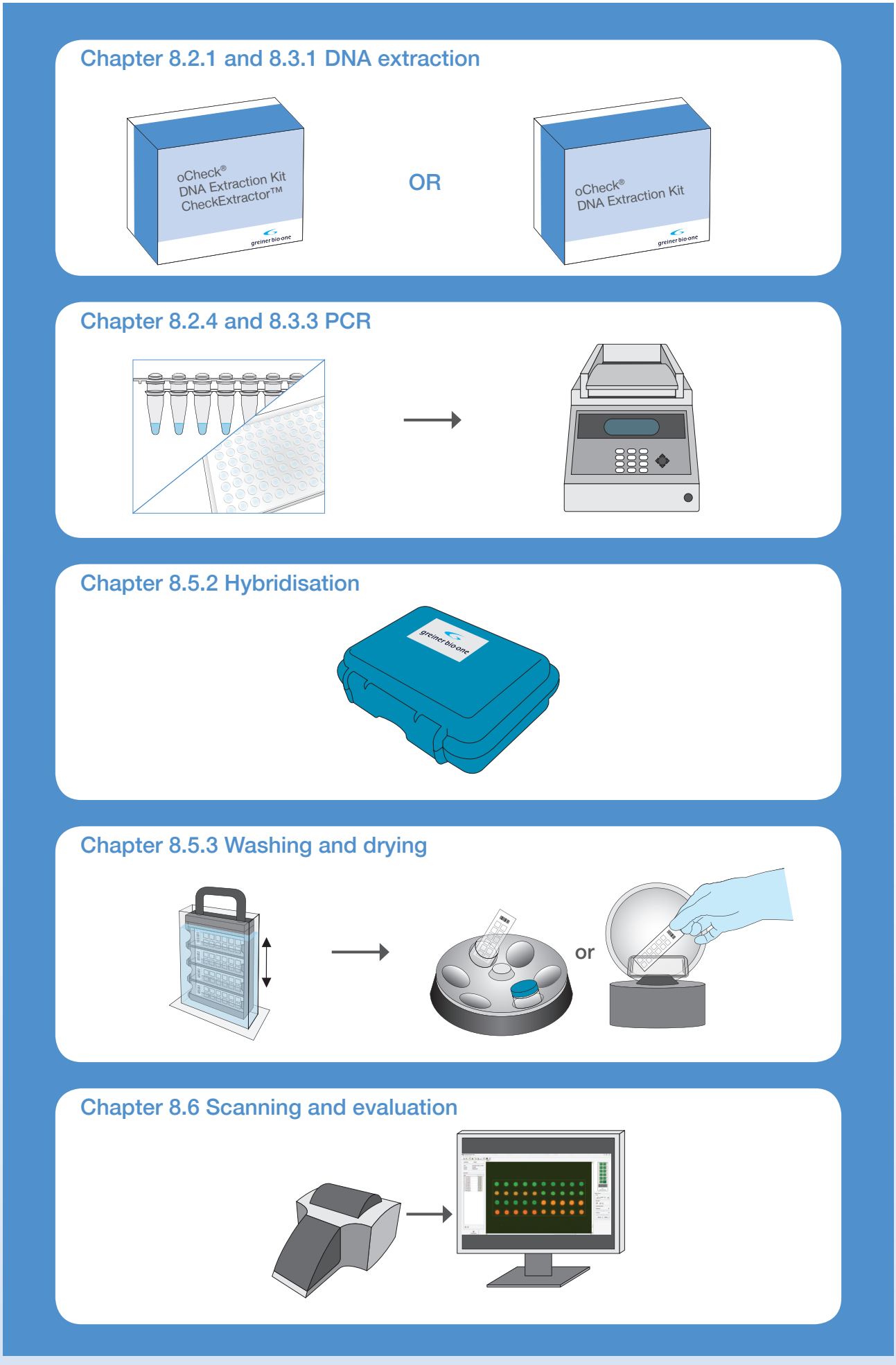


Figure 5: Overview of the different Pelvo**Check**® CT/NG working steps

8.2 Manual Processing

8.2.1 Manual DNA extraction

DNA extraction is not part of the Pelv**oCheck**® test kit. Extraction of DNA prior to the Pelv**oCheck**® CT/NG analysis must be performed using the oCheck® DNA Extraction Kit, also provided by Greiner Bio-One (see ordering information in chapter 2). Please follow the Instructions For Use carefully when using the oCheck® DNA Extraction Kit. The Pelv**oCheck**® CT/NG test kit is designed to process batches of 48 or 96 samples. It is not intended to process batches smaller than 48 samples.

Human urine samples or genital swabs collected with one of the following collection systems:

- Pelv**oCheck**® Collection Kit (Greiner Bio-One, Cat. No. 453 100 or 453 101) or
- Pelv**oCheck**® Swab Collection Kit (Greiner Bio-One, Cat. No. 453 103)

can be processed directly.

Samples collected with different collection systems or media may be treated differently before DNA extraction. For a detailed description on the sample preparation and DNA extraction procedure, please follow carefully the Instructions For Use for the oCheck® DNA Extraction Kit.

For further information on DNA extraction, please contact your local Greiner Bio-One distributor or consult the Greiner Bio-One website:
https://www.gbo.com/en_INT/know-how-services/download-center.htmL

8.2.2 Pooling

The Pelv**oCheck**® CT/NG test kit has also been validated for the analysis of up to five pooled urine samples. Pooling of the collected samples is accomplished before DNA extraction by merging five samples of 200 µl volume (Please note the lower sample volume compared to the analysis of single samples!). The DNA extraction from pooled samples must be performed using the oCheck® DNA Extraction Kit, but requires complementing Proteinase K and Collection Tubes. Please carefully follow the instructions described in detail in the Support Protocol for pooled urine samples for Pelv**oCheck**® CT/NG supplementing the Instructions For Use of the oCheck® DNA Extraction Kit.

8.2.3 External Quality Control for monitoring CT/NG performance

Sampling, DNA extraction and amplification are monitored by the internal controls Sample control and PCR control of Pelv**oCheck**® CT/NG (see Chapter 6.3.2. On-Chip Controls). For pathogen specific performance controlling, the addition of defined external run controls is required.
As pathogen-specific quality controls, AcroMetrix® CT/NG control (Life technologies, Cat.No. 967 146) is recommended. AcroMetrix® CT/NG control contains inactivated *Chlamydia trachomatis* and *Neisseria gonorrheae* bacterial cells in a proprietary matrix. The quality controls mimic natural occurring pathogens, and though they are inactivated, they are intact bacterial cells and allow the verification of bacterial nucleic acid extraction, purification and detection with Pelv**oCheck**® CT/NG.
Therefore, analyses can be performed according to the same instructions as for patient samples[17].

! For more detailed information concerning AcroMetrix® CT/NG control (Life technologies, Cat. No. 967 146), please refer to the product information. For ordering information, see Chapter 2.

[17] AcroMetrix® CT/NG Control Document Number MAN0007487 Effective Date 31 Dec 13 Rev A.00

AcroMetrix® CT/NG contains 1x 20 mL control material. For usage as Pelv**oCheck**® CT/NG quality controls, it is recommended to prepare 25 µL ready to use aliquots and store them at -20 °C or below. Do not refreeze aliquots and discard leftover of aliquots. Do not use AcroMetrix® CT/NG beyond the expiration date printed on the bottle.

In case of processing controls for individual samples, prepare 230.0 µL of the particular specimen collection medium and add 20.0 µL AcroMetrix® CT/NG Control to a final volume of 250.0 µL. Proceed according Instructions for Use for oCheck® DNA Extraction Kit/Single Column Preparation and subsequent Instructions for Use of Pelv**oCheck**® CT/NG (follow Chapter 8).

In case of processing controls for pooling samples, prepare 980.0 µL collection medium of the Pelv**oCheck**® Collection Kit SAFE or STRAW diluted 1:3,5 with PCR-grade water and add 20.0 µL AcroMetrix® CT/NG Control to a final volume of 1000.0 µL. Proceed according Instructions for Use for pooling samples extracted with oCheck® DNA Extraction Kit/Single Column Preparation and subsequent Instructions for Use of Pelv**oCheck**® CT/NG (follow Chapter 8).

If sample preparation, extraction, amplification, hybridisation and scanning were performed successfully, CheckReport™Software will evaluate the analyses of the external quality control as valid and positive for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

!

If the quality controls are needed in course of analysis of swab samples, it is recommended to use the specimen collection medium of a new Pelv**oCheck**® CT/NG Swab Collection Kit (REF 453 103).

In case of processing urine samples, it is recommended to use the specimen collection medium of a new Pelv**oCheck**® Collection SAFE or STRAW (REF 453 100 and 453 101) diluted 1:3,5 with PCR-grade water (e.g. Individual samples: 80 µL specimen collection medium + 200 µL PCR-grade water, Pooling samples: 320 µL specimen collection medium + 800 µL PCR-grade water) to perform positive controls with AcroMetrix® CT/NG Control.

If the use of specimen collection media as mentioned above is not possible, PCR-grade water can be used instead of the specimen collection media to perform specific performance controls. For pooling samples, the use of the Pelv**oCheck**® Collection SAFE or STRAW is mandatory.

8.2.4 Polymerase chain reaction (PCR)

The PCR is a very sensitive method which can detect extremely small amounts of DNA. Special precautions must be observed in order to avoid reaction contamination (see Chapter 7). HotStarTaq® DNA Polymerase and Uracil-N-Glycosylase are required but not provided with the Pelv**oCheck**® CT/NG test kit and must be separately purchased (see Chapter 2).

!

The Pelv**oCheck**® CT/NG test kit has been validated using HotStarTaq® DNA Polymerase from Qiagen and Uracil-N-Glycosylase from Fermentas (see ordering information in Chapter 2). It is mandatory to use these enzymes in order to achieve the claimed performance.

8.2.5 Thermal cycler setup

Pelv**oCheck**® CT/NG test kit has been validated with the following thermal cycler:

- GeneAmp® PCR system 9700 (Applied Biosystems)
- Veriti™ 96-Well Thermal Cycler (Applied Biosystems

!

It is absolutely necessary to use one of the thermal cyclers mentioned above in order to achieve the claimed performance.

The thermal cycler program of the PelvoCheck® CT/NG PCR is summarised in Table 1.

Table 1: Thermal cycler program of the PelvoCheck® CT/NG PCR

Time	Temp. °C	No. of cycles
20 min	37 °C	1 (UNG-Hydrolysis)
15 min	95 °C	1
30 s 60 s 30 s	94 °C 65 °C 72 °C	45
10 min	72 °C	1
Hold	10 °C	∞

In addition, the following run parameters must be set for each thermal cycler.

Set the reaction volume to 26 µl, the ramp speed to "9600" or "Max" and use a lid temperature of 103 °C. A higher heat ramp may cause an insufficient performance of the PCR reaction.

For a description on how to set these parameters see the Instructions For Use of the thermal cycler.

8.2.6 Uracil-N-Glycosylase (UNG) treatment[18]

The PelvoCheck® CT/NG PCR MasterMix contains dUTP, which is incorporated into the amplification products during the PelvoCheck® CT/NG PCR, rendering the PCR products susceptible to degradation by UNG. UNG cleaves the PCR product at sites where a deoxyuridylate residue has been incorporated. Cleaved PCR products will not be amplified in a subsequent reaction. Hence, an UNG treatment can be utilised to eliminate carry-over contamination from previous PCR reactions[19].

! The incorporated UNG system of the PelvoCheck® CT/NG PCR will only eliminate carry-over contamination with PCR products from previous PCR reactions. Other contamination, for example occurring during sample preparation, DNA extraction or PCR template addition, cannot be eliminated. Therefore it is still necessary to follow the instructions and special precautions for preventing contamination described in Chapter 7.

! The PelvoCheck® CT/NG test kit has been validated using Uracil-N-Glycosylase from Fermentas (see Chapter 2). It is mandatory to use this glycosylase to achieve the claimed performance.

- Dilute the Uracil-N-Glycosylase 1:50 in PCR-grade water (end concentration is 0.02 U/µL). Use a fresh UNG dilution for each PelvoCheck® CT/NG PCR reaction setup (see Chapter 8.2.7). Do not reuse the diluted UNG.
- Mix the UNG dilution carefully by either vortexing for 2 seconds and then spinning down or by pipetting up and down several times.
- Add 1 µL of this dilution to each PelvoCheck® CT/NG PCR reaction (see Chapter 8.2.7, Table 2).

[18] Purchase of PelvoCheck® CT/NG is accompanied by a limited license under U.S. Patent Numbers 5,035,996; 5,683,896; 5,945,313; 6,287,823; and 6,518,026 and corresponding foreign patents.

[19] Longo, M.C., et al. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions, Gene, 93, 125-128.

This amount is sufficient to eliminate PCR carry-over contamination. Take care not to use a higher concentrated UNG solution since this might have an adverse effect on PCR performance, resulting in a reduced sensitivity of PelvoCheck® CT/NG.

In general, for UNG treatment the final reaction mix is incubated for 20 minutes at 37 °C. Subsequently the UNG is inactivated by an additional incubation step of 15 minutes at 95 °C. These two steps are already incorporated into the Pelv**oCheck**® CT/NG PCR and correspond to the first two steps of the thermal cycler program (see Table 1). Within the second step (15 minutes at 95 °C) both inactivation of the Uracil-N-Glycosylase and activation of the HotStarTaq® DNA Polymerase occur.

8.2.7 PCR reaction setup

With the exception of the HotStarTaq® DNA Polymerase and the Uracil-N-Glycosylase the Pelv**oCheck**® CT/NG PCR MasterMix already contains all components necessary for performing the PCR reaction (PCR buffer, $MgCl_2$, dNTPs, primers, PCR control template).

The PelvoCheck® CT/NG test kit has been validated using HotStarTaq® DNA Polymerase from Qiagen (see Chapter 2). It is mandatory to use this DNA polymerase to achieve the claimed performance.

The setup of the final PCR MasterMix is optimally performed in a protected surrounding, e.g. a PCR hood, to avoid reaction contamination.

- Prepare the final PCR Master Mix in the MasterMix preparation tube(s) (consisting of Pelv**oCheck**® CT/NG PCR MasterMix, HotStarTaq® DNA Polymerase and Uracil-N-Glycosylase) for the required quantity (either 48 or 96) of PCR reactions as outlined in Table 2.

To analyse multiple samples, the final PCR MasterMix should be prepared in a batch (in the quantity required for all analyses). To adjust for volume variations during pipetting, it is recommended to increase the number of reactions (n) by 1 for each chip (= n+1), e.g. prepare a final PCR MasterMix volume for 13 amplification reactions if 12 samples are to be tested (see Table 2).

It is recommended to include a negative control for each batch of PelvoCheck® CT/NG PCR MasterMix prepared. As negative control, the DNA elution buffer of the appropriate DNA extraction kit or PCR-grade water may be used.

Always use one vial of PelvoCheck® CT/NG PCR MasterMix for the reactions of one chip.

- Mix the final PCR MasterMix thoroughly by either vortexing for 2 seconds and then spinning down or by pipetting up and down several times.
- Aliquot the final PCR MasterMix by pipetting 21 µL of the mix for each PCR reaction into a 0.2 mL thin-walled reaction tubes, or a 96-well PCR plate.

Carry out addition of the template DNA in a separate work space than the setup of the final PCR MasterMix (see Chapter 7.2).

- For the preparation of the final reaction mix, add 5 µL of DNA extract to each reaction mix and mix either by vortexing for 2 seconds and then spinning down or by pipetting up and down several

times. The total volume of one reaction mix is 26 µL.

➠ Place the reaction tubes, or the PCR plate in the thermal cycler and start the PCR reaction using the thermal cycler program described in chapter 8.2.5 Table 1 or chapter 8.3.4 Table 3.

! After the PCR has been completed, the amplification products should be used immediately for hybridisation or stored in the dark at ≤ -20 °C for up to three months, as the activity of the Uracil-N-Glycosylase can be partially restored at temperatures below 55 °C.

Table 2: Setup of the final PCR MasterMix

	52 reactions (sufficient for 48 reactions)	104 reactions (sufficient for 96 reactions)
Pelv**oCheck**® CT/NG PCR MasterMix	1034.8 µL	2069.6 µL
HotStarTaq® DNA Polymerase (5 U/µL)	5.2 µL	10.4 µL
Uracil-N-Glycosylase (Dilution of 1:50, 0.02 U/µL)	52 µL	104 µL
Total volume	1092 µL	2184 µL

8.3 Automated Processing using CheckExtractor™

8.3.1 Automated DNA extraction

DNA extraction is not part of the Pelv**oCheck**® CT/NG test kit. Pelv**oCheck**® CT/NG has been validated using DNA prepared with the Greiner Bio-One Check Extractor™, using the oCheck® DNA Extraction Kit – CheckExtractor™ from human urine and from vaginal and cervical swabs (see ordering information in Chapter 2). Please follow the Instructions For Use carefully when using the Greiner Bio-One CheckExtractor™ together with the oCheck® DNA Extraction Kit – CheckExtractor™. The Pelv**oCheck**® CT/NG test kit (REF 504 288) is designed to process batches of 48 or 96 samples. It is not intended to be used to process batches smaller than 48 samples.

8.3.2 External Quality Control for monitoring CT/NG performance

Sampling, DNA extraction and amplification are monitored by the internal controls Sample control and PCR control of Pelv**oCheck**® CT/NG (see Chapter 6.3.2. On-Chip Controls). For pathogen specific performance controlling, the addition of defined external run controls is required.

As pathogen-specific quality controls, AcroMetrix® CT/NG Control (Life technologies, Cat.No. 967146) is recommended. AcroMetrix® CT/NG Control contains inactivated *Chlamydia trachomatis* and *Neisseria gonorrhoeae* bacterial cells in a proprietary matrix. The quality controls mimic natural occurring pathogens, and though they are inactivated, they are intact bacterial cells and allow the verification of bacterial nucleic acid extraction, purification and detection with Pelv**oCheck**® CT/NG. Therefore, analyses can be performed according to the same instructions as for patient samples[20].

! For more detail information concerning AcroMetrix® CT/NG Control (Life technologies, Cat.No. 967146), please refer to the product information. For ordering information, see Chapter 2.

[20] AcroMetric® CT/NG Control Document Number MAN0007487 Effective Date 31 Dec 13 Rev A.00

AcroMetrix® CT/NG Control contains 1 x 20 mL control material. For usage as Pelv**oCheck**® CT/NG quality controls, it is recommended to prepare 25.0 µL ready to use aliquots and store them at ≤ - 20 °C. Do not refreeze aliquots and discard leftover of aliquots. Do not use AcroMetrix® CT/NG Control beyond the expiration date printed on the bottle.

In case of automated processing of swab samples, prepare 828.0 µL of the Pelv**oCheck**® Swab Collection Kit and add 72.0 µL AcroMetrix® CT/NG Control to a final volume of 900.0 µL in the sample tube for genital samples and proceed according to the Instructions for Use for the oCheck® DNA Extraction Kit - CheckExtractor™ and subsequent instructions in this manual (Chapter 8).

In case of automated processing of urine samples, prepare 1380.0 µL of Pelv**oCheck**® Collection medium (diluted 1:3,5 with PCR-grade water) and add 120.0 µL AcroMetrix® CT/NG Control to a final volume of 1500.0 µL in a Vacuette Tandem Tube (either gained from the Pelv**oCheck**® Collection Kit SAFE or STRAW or purchased separately, see ordering information in Chapter 2) and proceed according to the Instructions for Use for the oCheck® Extraction Kit - CheckExtractor™ and subsequent instructions in this manual (Chapter 8).

If sample preparation, extraction, amplification, hybridisation and scanning were performed successfully, CheckReport™Software will evaluate the analysis of the external quality control as valid and positive for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

! If the quality controls are needed in course of analysis of swab samples, it is mandatory to use the specimen collection medium of a new Pelv**oCheck**® CT/NG Swab Collection Kit (REF 453 103).

! In case of automated processing of urine samples, it is mandatory to use the specimen collection medium of a new Pelv**oCheck**® Collection SAFE or STRAW (REF 453 100 and 453 101) diluted 1:3,5 with PCR-grade water (e.g. 450 µL specimen collection medium + 1125 µL PCR-grade water) to perform positive controls with AcroMetrix® CT/NG Control.

8.3.3 Polymerase chain reaction (PCR)

The PCR is a very sensitive method which can detect extremely small amounts of DNA. Special precautions must be observed in order to avoid reaction contamination (see Chapter 7). HotStarTaq® DNA Polymerase and Uracil-N-Glycosylase are required but not provided with the Pelv**oCheck**® CT/NG test kit and must be separately purchased (see Chapter 2).

! The Pelv**oCheck**® CT/NG test kit has been validated using HotStarTaq® DNA Polymerase from Qiagen and Uracil-N-Glycosylase from Fermentas (see ordering information in Chapter 2). It is mandatory to use these enzymes in order to achieve the claimed performance.

8.3.4 Thermal cycler setup

Pelv**oCheck**® CT/NG test kit has been validated with the following thermal cycler:

- GeneAmp® PCR system 9700 (Applied Biosystems)
- Veriti™ 96-Well Thermal Cycler (Applied Biosystems

! It is absolutely necessary to use one of the thermal cyclers mentioned above in order to achieve the claimed performance.

The thermal cycler program of the Pelv**oCheck**® CT/NG PCR is summarised in Table 3.

Table 3: Thermal cycler program of the PelvoCheck® CT/NG PCR

Time	Temp. °C	No. of cycles
20 min	37 °C	1 (UNG-Hydrolysis)
15 min	95 °C	1
30 s 60 s 30 s	94 °C 65 °C 72 °C	45
10 min	72 °C	1
Hold	10 °C	∞

In addition, the following run parameters must be set for each thermal cycler.

Set the ramp speed to "9600" or "Max" and use a lid temperature of 103 °C. A higher heat ramp may cause an insufficient performance of the PCR reaction.

For a description on how to set these parameters see the Instructions For Use of the thermal cycler.

8.3.5 Uracil-N-Glycosylase (UNG) treatment[21]

The PelvoCheck® CT/NG PCR MasterMix contains dUTP, which is incorporated into the amplification products during the PelvoCheck® CT/NG PCR, rendering the PCR products susceptible to degradation by UNG. UNG cleaves the PCR product at sites where a deoxyuridylate residue has been incorporated. Cleaved PCR products will not be amplified in a subsequent reaction. Hence, an UNG treatment can be utilised to eliminate carry-over contamination from previous PCR reactions[22].

! The incorporated UNG system of the PelvoCheck® CT/NG PCR will only eliminate carry-over contamination with PCR products from previous PCR reactions. Other contamination, for example occurring during sample preparation, DNA extraction or PCR template addition, cannot be eliminated. Therefore it is still necessary to follow the instructions and special precautions for preventing contamination described in Chapter 7.

! The PelvoCheck® CT/NG test kit has been validated using Uracil-N-Glycosylase from Fermentas (see Chapter 2). It is mandatory to use this glycosylase to achieve the claimed performance.

- Dilute the Uracil-N-Glycosylase 1:50 in PCR-grade water (end concentration is 0.02 U/µL). Use a fresh UNG dilution for each PelvoCheck® CT/NG PCR reaction setup (see Chapter 8.3.6). Do not reuse the diluted UNG.
- Mix the UNG dilution carefully by either vortexing for 2 seconds and then spinning down or by pipetting up and down several times.
- Add 1 µL of this dilution to each PelvoCheck® CT/NG PCR reaction (see Chapter 8.3.6, Table 4).

[21] Purchase of PelvoCheck® CT/NG is accompanied by a limited license under U.S. Patent Numbers 5,035,996; 5,683,896; 5,945,313; 6,287,823; and 6,518,026 and corresponding foreign patents.

[22] Longo, M.C., et al. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions, Gene, 93, 125-128.

This amount is sufficient to eliminate PCR carry-over contamination. Take care not to use a higher concentrated UNG solution since this might have an adverse effect on PCR performance, resulting in a reduced sensitivity of PelvoCheck® CT/NG.

In general, for UNG treatment the final reaction mix is incubated for 20 minutes at 37 °C. Subsequently the UNG is inactivated by an additional incubation step of 15 minutes at 95 °C. These two steps are already incorporated into the Pelv**oCheck**® CT/NG PCR and correspond to the first two steps of the thermal cycler program (see Table 1). Within the second step (15 minutes at 95 °C) both inactivation of the Uracil-N-Glycosylase and activation of the HotStarTaq® DNA Polymerase occur.

8.3.6 PCR reaction setup

With the exception of the HotStarTaq® DNA Polymerase and the Uracil-N-Glycosylase the Pelv**oCheck**® CT/NG PCR MasterMix already contains all components necessary for performing the PCR reaction (PCR buffer, $MgCl_2$, dNTPs, primers, PCR control template).

The PelvoCheck® CT/NG test kit has been validated using HotStarTaq® DNA Polymerase from Qiagen (see Chapter 2). It is mandatory to use this DNA polymerase to achieve the claimed performance.

The setup of the final PCR MasterMix is optimally performed in a protected surrounding, e.g. a PCR hood, to avoid reaction contamination.

- Prepare the final PCR MasterMix in the MasterMix Preparation tube(s) (consisting of Pelv**oCheck**® CT/NG PCR MasterMix, HotStarTaq® DNA Polymerase and Uracil-N-Glycosylase). For 48 analyses prepare 1 MasterMix preparation tube and for 96 analyses prepare 2 MasterMix preparation tubes as outlined in Table 4.

The kit compilation has been developed to use 1 MasterMix preparation tube for 48 analyses and 2 MasterMix preparation tubes for 96 analyses. To adjust for volume variations during pipetting, the final MasterMix contains reagents sufficient for 58 analyses.

It is recommended to include a negative control for each batch of final PCR MasterMix prepared. As negative control, the DNA elution buffer of the appropriate DNA extraction kit or PCR-grade water may be used.

- Mix the final PCR MasterMix thoroughly by either vortexing for 2 seconds and then spinning down or by pipetting up and down several times.

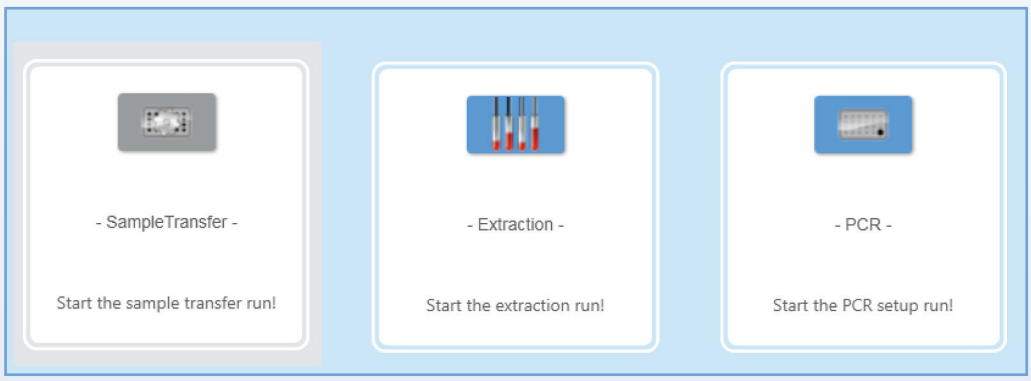
Table 4: Setup of the final PCR MasterMix

	58 reactions (sufficient for 48 reactions)	116 reactions (sufficient for 96 reactions)
PelvoCheck® CT/NG PCR MasterMix	1154.2 µL	2308.4 µL
HotStarTaq® DNA Polymerase (5 U/µL)	5.8 µL	11.6 µL
Uracil-N-Glycosylase (Dilution of 1:50, 0.02 U/µL)	58 µL	116 µL
Total volume	1218 µL	2436 µL

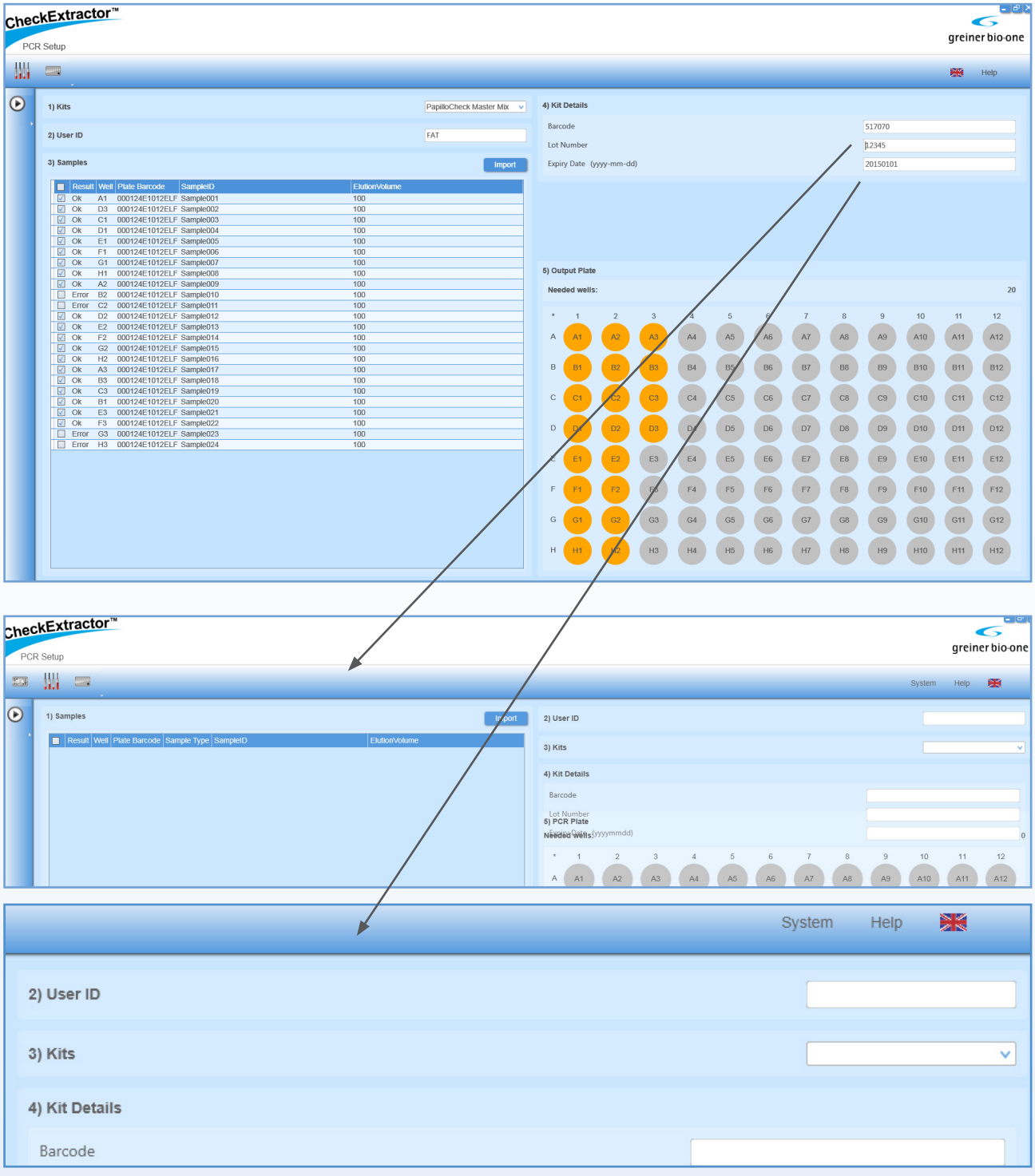
The volume for 1 PCR reaction is 26 µL consisting of 21 µL final PCR MasterMix and 5 µL DNA template.

- On the start screen, chose “PCR” to start the PCR Setup method.
- Switch on the PC and the CheckExtractor™.
- Log in as a lab operator.
- Double-click the Start icon on the PC desktop.

- Check the fill level of the liquid waste bottle. Follow the instructions of the CheckExtractor™ Software, if emptying is necessary.
- Select „PCR“ to start the PCR setup run



A screen asking for basic information will appear.

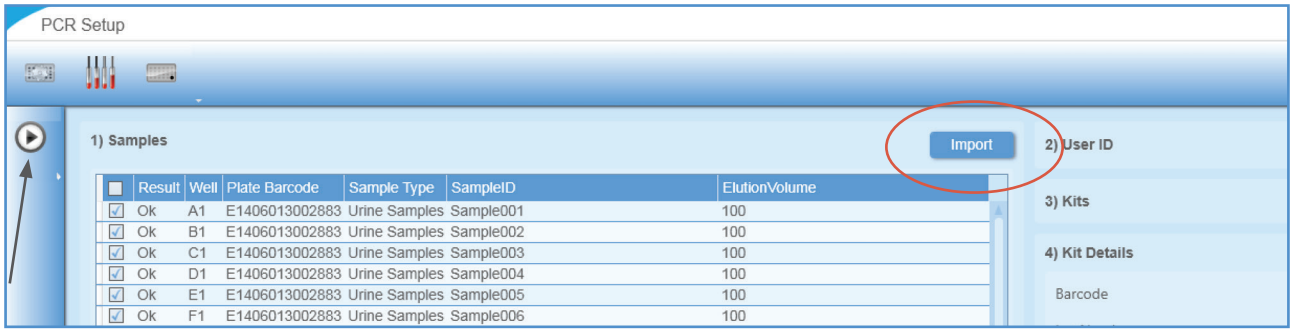


➠ Complete the Run information:

1. Insert **USER ID**, type of protocol, sample and labware type to complete run information
2. press **Import** in the upper right corner to start
3. Choose eultion plate to be processed under "**1) Samples**" in the upper left corner

Please make sure to choose the correct file by checking the file name regarding barcode and exact issuing date. If the elution plate has already been used for a PCR setup you have to import the file with the latest issuing date.

After importing the data for a specific elution plate, a list of samples is displayed. By default, the CheckExtractor™Software will mark each sample, for which the DNA extraction was successfully performed, as active. The PCR setup will be performed for all active samples.



If the PCR setup should be performed for fewer samples, samples have to be deactivated by the user under "5) PCR Plate". Again, the needed consumables, namely tips, and final PCR MasterMix are calculated by the CheckExtractor™Software. Please follow exactly the instructions of the software during equipping the different carriers.

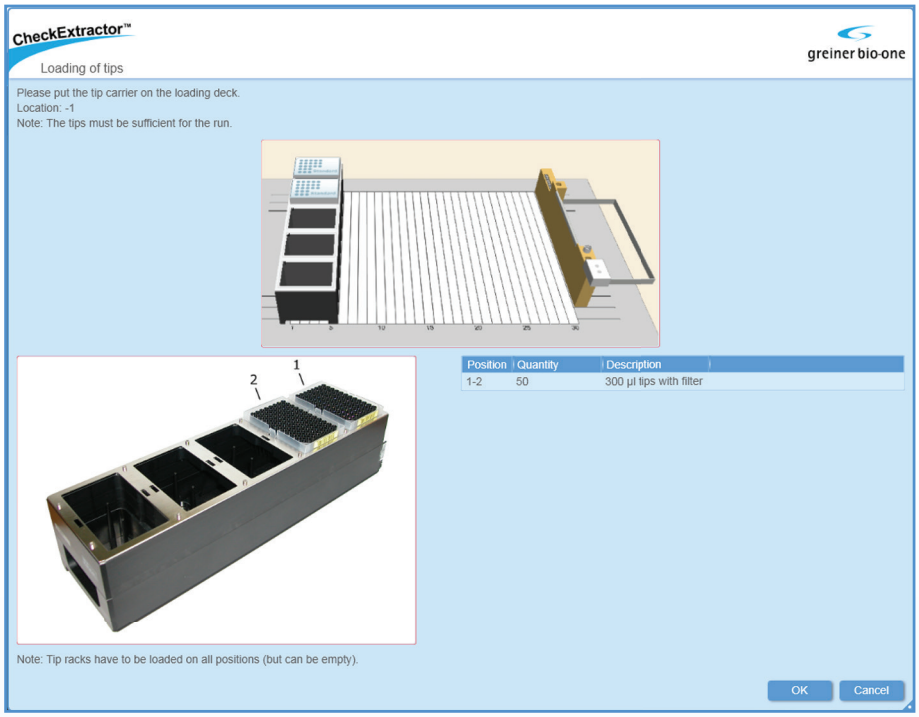
Next,

1. Fill in user ID under "**2) User ID**"
2. Select Pelv**oCheck**® CT/NG under "**3) Kits**" you can choose the appropriate assay. Depending on the samples you have imported you can choose between several assays. If you import genital samples you have the possibility to either choose Papill**oCheck**®, Papill**oCheck**® high-risk or Pelv**oCheck**® CT/NG. If you import urine samples just Pelv**oCheck**® CT/NG can be selected.
3. Complete the data under "**4) Kit details**", place the cursor into the white box for "**Barcode**". Scan the barcode of the Pelv**oCheck**® CT/NG kit to be used with the provided handheld scanner. The software will automatically fill that box with the reference number of the kit and the other two boxes with the Lot number and the expiry date of the kit.

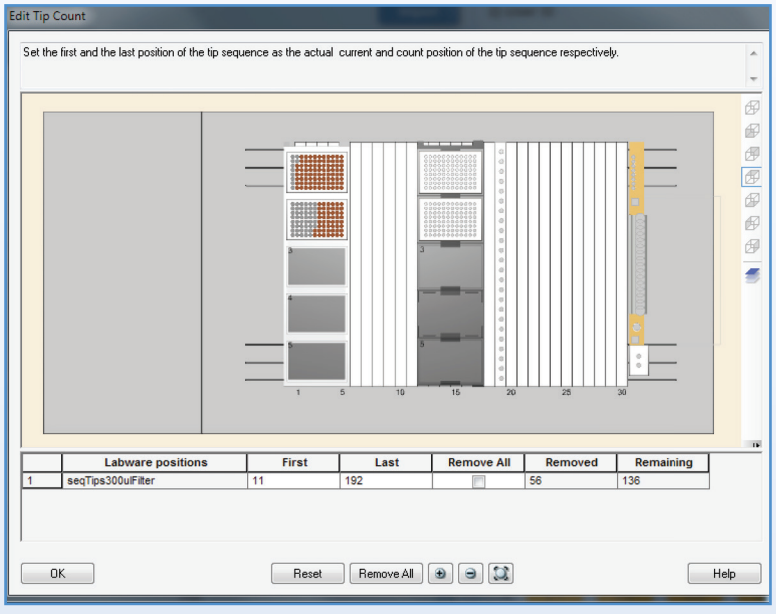
The barcode of the Pelv**oCheck**® CT/NG kit can be found in the upper left corner of the kit lid.

➠ Press the start button in the upper left corner of the screen to start the PCR-Setup run.

➠ A screen describing the loading of tips will appear.



- Equip the tip carriers with tips. Make sure to use the correct carrier positions.
- Place the tip carriers on the loading deck as displayed starting at track 1. The carriers have to be threaded on the deck and pushed forward until stop.
- Press the "**OK**" button. The CheckExtractor™ will load the carriers automatically.
- The Tip Editor will appear.



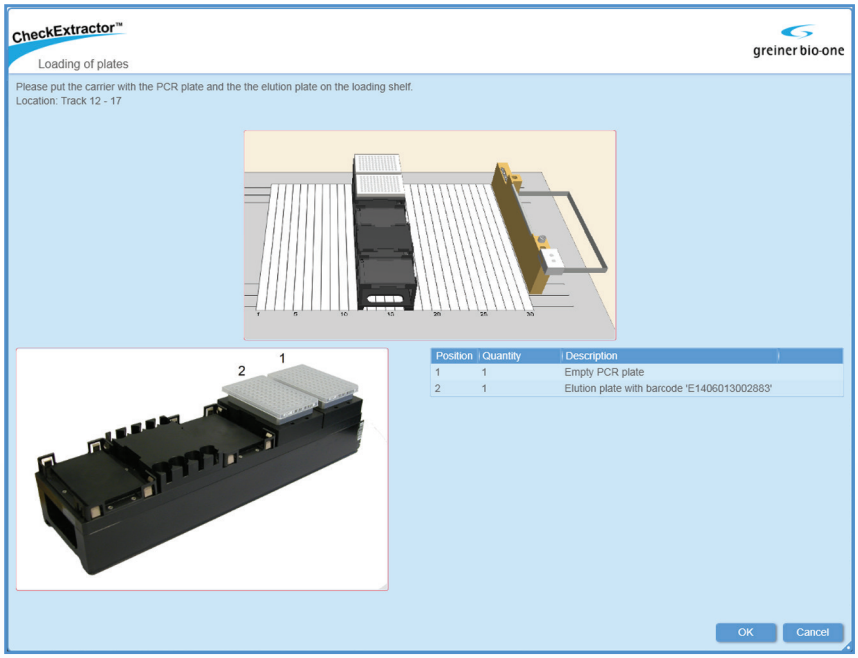
Please check whether the displayed tip setting is correct. Tips still available for the procedure are displayed in brown. Empty tip positions are displayed grey.

- If the tip setting is displayed correctly, press the "**OK**" button.

!

If the tip setting has to be adjusted, please refer to the CheckExtractor™ Instructions For Use for an explanation on how to use the tip editor.

A screen describing the loading of plates will appear.



- Equip the plate carrier with an empty barcoded PCR-plate and the elution plate to be processed. Place the PCR-plate in position 1 of the carrier and the elution plate in position 2. The barcodes of both plates have to face to the right and therefore in the direction of the barcode reader.

> **!** For an optimal laboratory workflow and to avoid unnecessary freeze and thaw cycles, it is recommended that the PCR setup run is directly performed after the extraction run has finished. In this case the elution plate of the previous extraction run is still placed in position 2 and only the empty PCR plate has to be added.

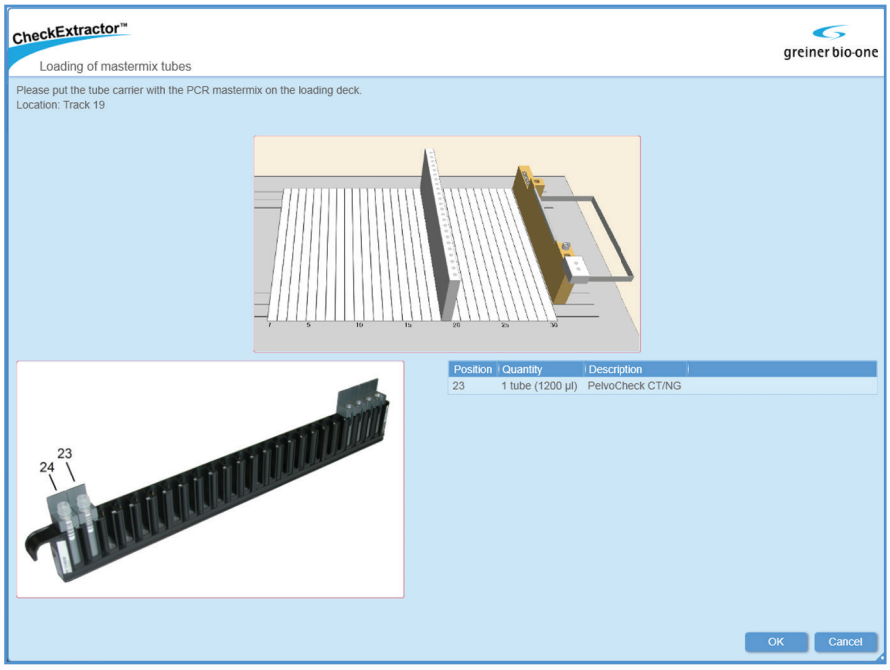
> **!** If for the PCR setup run an "older" elution plate is used, which was probably sealed to be stored, make sure to thaw the plate, spin down briefly using a plate centrifuge and remove the sealing before placing it onto the plate carrier (for a detailed description on the sealing procedure refer to the CheckExtractor™ IFU).

- Place the plate carrier on the loading deck using tracks 12-17. The carriers have to be threaded on the deck and pushed forward until stop.
- Press the "**OK**" button. The CheckExtractor™ will load the carrier automatically.

> **!** To make sure that the carrier will be loaded by the CheckExtractor™, place the carrier on the deck and cautiously push the carrier into the right track until a light mechanical stop is noticeable. From that position the carrier will be loaded automatically after confirming with "OK".

> **!** The barcode of the used elution plate has to fit to the plate chosen before under "1) Samples" on the run definition screen. Otherwise an error message will be displayed.

A screen describing the loading of the MasterMix preparation tube will appear.



- Equip the tube carrier with the barcoded MasterMix preparation tube(s). Make sure that the adapters are placed correctly. For a PCR setup of up to 48 samples, place one barcoded tube filled with prepared final PCR MasterMix into the adapter at carrier position 23. For a PCR setup of up to 96 samples, place two filled tubes into the tube carrier. Use adapters at carrier positions 23 and 24. Make sure that the barcodes face to the right and therefore in the direction of the barcode reader.

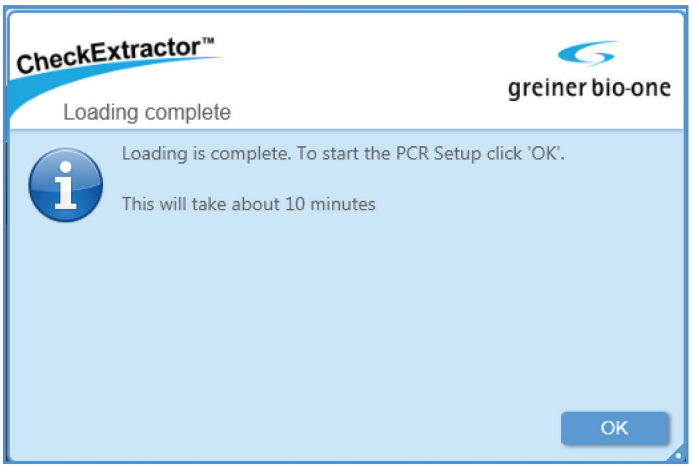
> **!** If the solution is mixed by vortexing and therefore the included cap of the barcoded vial is used, this cap has to be removed before placing the vial into the adapters of the tube carrier.

> **!** Be aware that the CheckExtractor™ is designed to perform either 48 PCR set-ups or 96. Even if less samples are prepared for the PCR the MasterMix has to be prepared for either 48 or 96 samples.

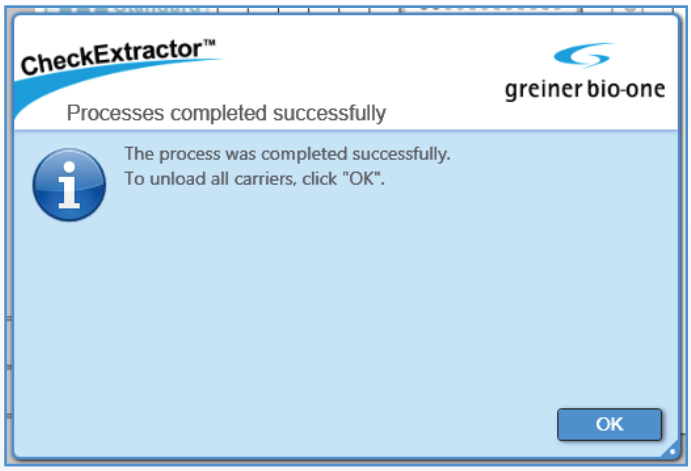
- Place the tube carrier on the loading deck using track 19. The carriers have to be threaded on the deck and pushed forward until stop.
- Press the "**OK**" button. The CheckExtractor™ will load the carrier automatically.

> **!** To make sure that the carrier will be loaded by the CheckExtractor™, place the carrier on the deck and cautiously push the carrier into the right track until a light mechanical stop is noticeable. From that position the carrier will be loaded automatically after confirming with "OK".

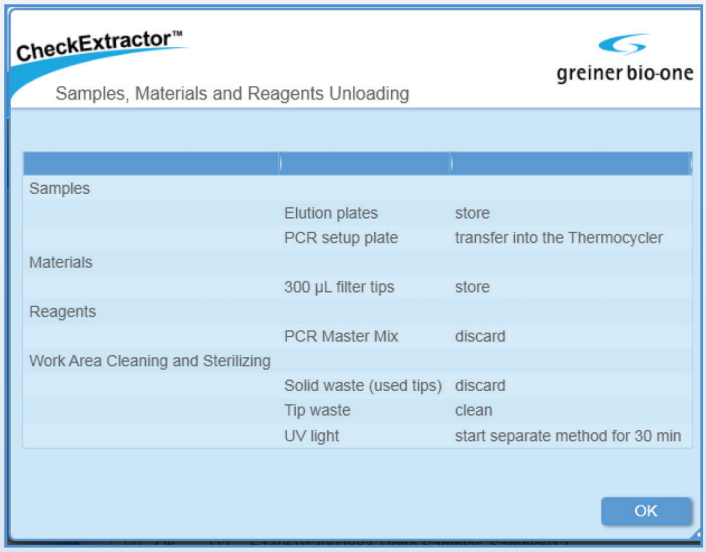
The loading is now complete. The CheckExtractor™ will display the estimated run time and will ask to confirm the start of the PCR Setup run.



- Click “**OK**” to start the PCR Setup run.
- At the end of a successful run, click ”**OK**” to start the unloading procedure and the cleaning of the deck.



After confirmation, the CheckExtractor™ will automatically unload all carriers. Please remove them from the loading deck. Please follow the further instructions in the correct order displayed on the screen to safely unload the CheckExtractor™.



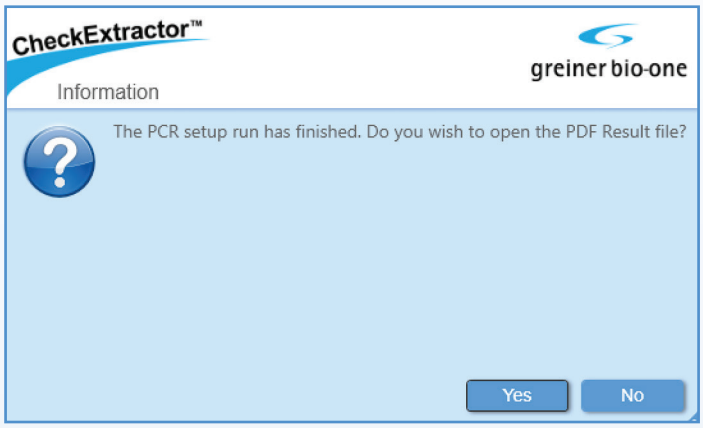
- Unload the elution plate from the plate carrier. Handle the plate carefully to avoid spilling over.

! For storage of the elution plate for further analyses or re-analyses, seal the elution plate with an adhesive foil (see chapter 8.4) and store at -20 °C.

- Unload the PCR plate with the reaction mix from the plate carrier. Handle the plate carefully to avoid spilling over. Seal the PCR plate using heat sealing as described in Chapter 8.4.
- Transfer the sealed plate to the PCR cycler and start the PCR reaction using the thermal cycler program described in 8.2.5 (Table 1) and in Chapter 8.3.4 (Table 3).

> **!** After the PCR has been completed, the amplification products should be used immediately for hybridisation or stored in the dark at -20 °C for up to one week.

- Store the complete tip carrier with unused filter tips still on board.
- Discard the barcoded MasterMix vials.
- Discard the used tips in the solid laboratory waste.
- Clean all the carriers and the tip waste with the recommended cleaning solution (for more information consult the CheckExtractor™ Instructions for Use).
- Start the UV light method for 30 minutes to additionally support DNA decontamination of the CheckExtractor™.
- Confirm the complete clean-up of the deck by clicking "**OK**". By that, the CheckExtractor™ ask whether to open the PDF Result file or not. By clicking on "**Yes**" the PDF Result file will be open and by clicking on "**No**" you return to the start screen.



The exact sample configuration of the resulting PCR plate is included in the report files. This is the basis for the post PCR processing including hybridisation and washing.

> **!** Please be aware that the layout may be different to the one of the used elution plate, as samples for which the extraction did not work and deactivated samples are skipped. This results in potentially not used wells at the last positions of the PCR plate.

DateAndTime	2015-03-02 11:47:33
NumberOfWells	96
SampleType	Urine Samples
RunID	bfdd846dff924466a04d96a905934dc7
UserID	MiS ER
WindowsUserName	technical_support
InstrumentID	6665
PCRSetupKitBarcode	PEK288
PCRSetupKitLotNumber	15001
PCRSetupKitExpiryDate	2015-12-30
PCRSetupKit	PelvoCheck CT/NG
ElutionPlateBarcode	E1406013002883
PCRPlateBarcode	E1406013002854

greiner bio-one

PCR Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample001 1 Ok	Sample009 9 Ok	Sample017 17 Ok	Sample025 25 Ok	Sample033 33 Ok	Sample041 41 Ok	Sample049 49 Ok	Sample057 57 Ok	Sample065 65 Ok	Sample073 73 Ok	Sample081 81 Ok	Sample089 89 Ok
B	Sample002 2 Ok	Sample010 10 Ok	Sample018 18 Ok	Sample026 26 Ok	Sample034 34 Ok	Sample042 42 Ok	Sample050 50 Ok	Sample058 58 Ok	Sample066 66 Ok	Sample074 74 Ok	Sample082 82 Ok	Sample090 90 Ok
C	Sample003 3 Ok	Sample011 11 Ok	Sample019 19 Ok	Sample027 27 Ok	Sample035 35 Ok	Sample043 43 Ok	Sample051 51 Ok	Sample059 59 Ok	Sample067 67 Ok	Sample075 75 Ok	Sample083 83 Ok	Sample091 91 Ok
D	Sample004 4 Ok	Sample012 12 Ok	Sample020 20 Ok	Sample028 28 Ok	Sample036 36 Ok	Sample044 44 Ok	Sample052 52 Ok	Sample060 60 Ok	Sample068 68 Ok	Sample076 76 Ok	Sample084 84 Ok	Sample092 92 Ok
E	Sample005 5 Ok	Sample013 13 Ok	Sample021 21 Ok	Sample029 29 Ok	Sample037 37 Ok	Sample045 45 Ok	Sample053 53 Ok	Sample061 61 Ok	Sample069 69 Ok	Sample077 77 Ok	Sample085 85 Ok	Sample093 93 Ok
F	Sample006 6 Ok	Sample014 14 Ok	Sample022 22 Ok	Sample030 30 Ok	Sample038 38 Ok	Sample046 46 Ok	Sample054 54 Ok	Sample062 62 Ok	Sample070 70 Ok	Sample078 78 Ok	Sample086 86 Ok	Sample094 94 Ok
G	Sample007 7 Ok	Sample015 15 Ok	Sample023 23 Ok	Sample031 31 Ok	Sample039 39 Ok	Sample047 47 Ok	Sample055 55 Ok	Sample063 63 Ok	Sample071 71 Ok	Sample079 79 Ok	Sample087 87 Ok	Sample095 95 Ok
H	Sample008 8 Ok	Sample016 16 Ok	Sample024 24 Ok	Sample032 32 Ok	Sample040 40 Ok	Sample048 48 Ok	Sample056 56 Ok	Sample064 64 Ok	Sample072 72 Ok	Sample080 80 Ok	Sample088 88 Ok	Sample096 96 Ok

8.4 Sealing

8.4.1 Sealing and storage of plates

In this chapter the sealing of the plates is described in order to store them at ≤ -20 °C.

> Nevertheless, it is recommended to perform the PCR immediately after the extraction.

8.4.1.1 Sealing and storage of the elution plate[23]

For storage at ≤ -20 °C, the elution plate has to be closed after unloading from the CheckExtractor™.

- Close the elution plate after removal from the device by attaching a Greiner Bio-One adhesive foil (Silver seal, 80.0 / 140 MM, REF 676 090). Press it firmly onto the surface of the plate. Store the plate at ≤ -20 °C.

> It is recommended to use the Greiner Bio-One adhesive foil (Silver seal, REF 676 090) for storage of the elution plate as it can easily be removed from the plate if the plate has to be reused for a PCR setup using the CheckExtractor™.
> But it is also possible to use the sealing procedure described for the PCR plate to close the elution plate.

8.4.1.2 Sealing of the PCR plate

In case of performing automated processing, the PCR plate must be sealed before transferring it to the PCR cycler after the unloading procedure of the CheckExtractor™.

[23] Only required when using CheckExtractor™ and performing automated processing.

In case of performing manual processing, the PCR reaction can be performed using 0.2 µL reaction tubes, 8 x 0.2 µL reaction tubes or a 96well PCR-plate. In case of usage of a 96well-PCR plate, the plate must be sealed as well before transferring it to the PCR cycler.
For this purpose, a sealing foil (Pierce Seal, REF 865 804) is welded onto the PCR plate using a heat sealer (Model: 4S3, REF 865 802). This procedure is described in Figure 6.

- Turn on the heat sealer using a switch at the rear part of the housing. Turn it on about 5 minutes prior to use to ensure that the required sealing temperature of 170 °C can be reached.
- Check the programmed sealing temperature and duration of the sealing procedure, by pressing the "**SET**" key (1x: the sealing temperature is displayed; 2x: the duration of the sealing procedure is displayed). For the correct sealing of the plate, a sealing temperature of **170 °C** and a sealing duration of t2.0 seconds must be set. If one of these values or both are not correct, please adjust the values using the "▲" and "▼" keys.

!

In order to adjust the sealing temperature, press the "SET" key in the top left corner of the front of the device. The currently set sealing temperature appears on the display and flashes. The required temperature can then be set using the "▲" and "▼" keys. By pressing again, the last visible sealing temperature is confirmed and the currently set duration of the sealing procedure is displayed (also flashing). The duration can now also be adjusted using the "▲" and "▼" keys. By pressing again the "SET" key, the duration time is confirmed and stored on the device.

- Wait until the device has reached a temperature of 170 °C (the actual temperature is constantly displayed). In addition to the display, this is also identifiable by a beep sound that is heard when the device is ready for use.
- Open the device by pressing the "**OPERATE**" key. The tray with insert for the PCR plate will open up automatically.
- Remove the metal frame and put the PCR plate onto the insert of the tray. Then place the sealing foil (Pierce Seal, REF 865 804) onto the PCR plate. Make sure that the blue line is facing side up and that all wells are well covered. Place the metal frame on the top of the plate.
- Close the device by pressing again the "**OPERATE**" key. The tray will then close automatically and the sealing procedure starts.
- After the sealing procedure the tray opens again automatically. First, remove the metal frame and then the sealed PCR plate.

!

After removal of the PCR plate, the device can be closed by pressing the "CLOSE" key. To avoid bending the metal frame it is recommended to keep the frame inside the device. When closed, the device can be turned off anytime, using the power switch at the rear part of the housing.

- Turn on the heat sealer 5 minutes prior to use.
- Use sealing temperature of 170 °C and duration of t2.0 seconds.
- Press “OPERATE” to open device.

- Remove the metal frame and put the PCR plate onto the insert of the tray.
- Place sealing foil (Pierce Seal, REF 865 804) onto the PCR plate.
- Place metal frame on top of the plate.
- Press “OPERATE”.
- Remove sealed plate, press “CLOSE”.
- Turn off sealer.

Figure 6: Sealing procedure using a heat sealer

8.4.1.3 Reclosing of the PCR plate

After performing the hybridisation, the PCR plate can be resealed and stored again at ≤ -20 °C.

- Close the PCR plate by attaching a Greiner Bio-One adhesive foil (Silver seal, REF 676 090). Press it firmly onto the surface of the plate. Store the plate at ≤ -20 °C.

! The adhesive foil is stuck directly onto the sealing film. When removing the foil, it is possible that the underlying sealing foil may partially detach from the plate. However, this is not a problem for taking out PCR products from the PCR plate.

- Place the PCR plate in the thermal cycler and start the PCR reaction using the thermal cycler program described in Chapter 8.2.5 (Table 1) and Chapter 8.3.4 (Table 3).

! After the PCR has been completed, the amplification products should be used immediately for hybridisation or stored in the dark at ≤ -20 °C for up to three months, as the activity of the Uracil-N-Glycosylase can be partially restored at temperatures below 55 °C.

8.5 Hybridisation and washing

8.5.1 Preparation and setup

Hybridisation must be performed at room temperature (18 to 25 °C). Begin with the necessary preparations for the hybridisation and washing steps at least 30 minutes in advance of starting the hybridisation procedure.

- To dissolve potential precipitates in the hybridisation and washing buffers, expose them to room temperature (18 to 25 °C) for 30 minutes and mix well before use.

Storage of the Pelv**oCheck**® CT/NG test kit at 4 to 8 °C may result in precipitation of salts in the Hybridisation Buffer and Buffer B. Allow the solutions to equilibrate to room temperature (18 to 25 °C) and then vortex the tube or mix the bottle until any precipitate is dissolved.

- Prepare the oCheck® Hybridisation Chamber: Put a fresh wet paper towel into the Hybridisation Chamber and close the lid to create a humidity-saturated atmosphere.

To avoid evaporation of the small volume of used hybridisation mix on the chip, it is necessary to perform the hybridisation in a humidity-saturated atmosphere. A dedicated Hybridisation Chamber for Pelv**oCheck**® CT/NG analysis is available from Greiner Bio-One (see ordering information in Chapter 2).

- Incubate the required amount of Pelv**oCheck**® CT/NG chips in the prepared hybridisation chamber at room temperature (18 to 25 °C) for at least 10 minutes.

!

The magnetic slide holder of the Hybridisation Chamber contains a magnet only at one of two ends. If less than four Pelv**oCheck**® CT/NG chips are to be hybridised in parallel, take care to fill the slideholder with PelvoCheck® CT/NG chips from the opposite side of the magnet. Otherwise, the Pelv**oCheck**® CT/NG chips will not be covered with liquid during the washing procedure.

Keep unused Pelv**oCheck**® CT/NG chips in the Pelv**oCheck**® CT/NG Slidebox and store it inside the closed plastic pouch with the desiccant bag as well as protected from light.

Preparation of washing solutions I and II:

- Prepare the washing solutions I and II appropriate for the number of Pelv**oCheck**® CT/NG chips being analysed as shown in Table 5.
- Aliquot 2 equal volumes of the washing solutions into two separate oCheck® Washboxes and label them as washing solution I and II. Each oCheck® Washbox contains an engraved scale, indicating the correct amount of washing solution needed for up to 4 chips. Please use this scale to check the buffer quantity.
- Preheat washing solution II to 50 °C in a temperature-controlled water bath for at least 20 minutes prior to use. Ensure that the fill level of the water bath equals the fill level of the washing solution II.

Table 5: Preparation of the washing solution mix

Components	Number of PelvoCheck® CT/NG chips
	4
Purified water	400 mL
PelvoCheck® CT/NG Buffer A	40 mL
PelvoCheck® CT/NG Buffer B	5 mL
Total volume	445 mL

The volumes summarised in this table are sufficient for both washing steps (washing solutions I and II) for the indicated number of Pelv**oCheck**® CT/NG chips.

!

Never reuse the washing solutions as this could lead to an accumulation of washed-off PCR product that possibly interferes with Pelv**oCheck**® CT/NG results. Use fresh washing solutions for each assay.

In advance prepared washing solution mix can be stored up to one week at room temperature (18 to 25 °C). Check if precipitation of SDS has occurred. If so, warm up the washing solution mix until the precipitate is dissolved and equilibrate to room temperature (18 to 25 °C) again. Then prepare for the next hybridisation experiment.

8.5.2 Hybridisation

Hybridisation must be performed at room temperature (18 to 25 °C). The principle working steps for hybridising the PCR products of the Pelv**oCheck**® CT/NG PCR reaction onto the Pelv**oCheck**® CT/NG chip are shown in Figure 7.

- Mix the PCR products before use. Briefly spin down.

!

If PCR products were stored at ≤ -20 °C until hybridisation, first thaw PCR products before mixing and then proceed as described.

- Vortex the Pelv**oCheck**® CT/NG Hybridisation Buffer before use. Briefly spin down.
- Mix 30 µL of the Pelv**oCheck**® CT/NG Hybridisation Buffer in a fresh reaction tube of an 8x PCR strip or a PCR plate with 10 µL of the PCR product by either vortexing or by pipetting up and down several times.
- Depending on the PCR setup method you were using (manual or automated) the PCR products can be also processed in a sealed PCR plate. In this case pierce the foil with the tips and remove the appropriate volume with new tips.
- Briefly spin down.
- Transfer 30 µL of the hybridisation mix into each chip well by using six channels of a multichannel pipette. Avoid air bubble formation!

It is recommended to process six samples in parallel using an 8-channel multipipette and 8x PCR strips or a PCR plate (see Figure 7). This increases handling efficiency, speed and thereby reduces the risk of evaporation. If more than one slide is to be processed at once, the usage of a multipipette is mandatory in order to achieve the correct hybridisation time.

If possible, hybridise all 12 wells of a chip. In case of processing fewer than 12 samples, leave the unused wells empty. Unused wells on a processed chip cannot be used for future samples.

!

Handle the chip carefully to avoid spilling of the hybridisation mix. Spilling can lead to cross-contamination of samples and to false positive results.

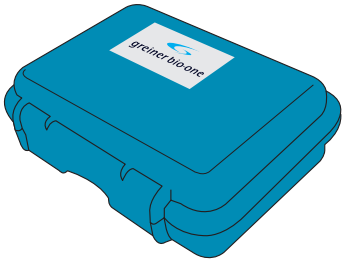
➠ Incubate the chip for exactly 30 minutes at room temperature (18 to 25 °C) within the prepared Hybridisation Chamber in a dark, humidity-saturated atmosphere. Be careful not to move the Hybridisation Chamber during the hybridisation.

!

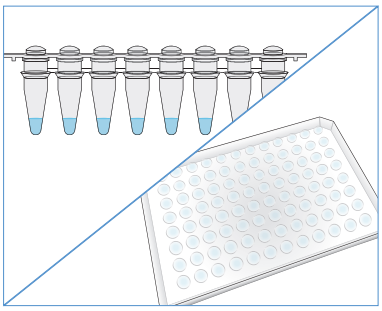
Never change the incubation time or temperature of the hybridisation reaction, as this may cause a loss of fluorescence signal intensity or an increase in unspecific fluorescence.

Do not expose the hybridised chips to direct sunlight.

- Prepare a humidity-saturated atmosphere in the Hybridisation Chamber.
- Incubate the required amount of Pelv**oCheck**® CT/NG chips in the Hybridisation Chamber at room temperature (18 to 25 °C) (see Chapter 8.5.1).



- Mix 30 µL of the Pelv**oCheck**® CT/NG Hybridisation Buffer in a 0.2 mL reaction tube of a PCR strip or a PCR plate with 10 µL of the PCR product. Mix thoroughly.



- Transfer 30 µL of hybridisation mix into each well of the Pelv**oCheck**® CT/NG chip using a multichannel pipette. Avoid air bubble formation!

- Close the Hybridisation Chamber and incubate the Pelv**oCheck**® CT/NG chip for exactly 30 minutes at room temperature (18 to 25 °C).

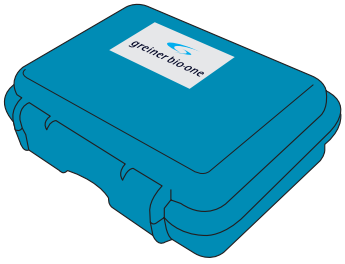


Figure 7: Working steps of the hybridisation procedure

8.5.3 Washing and drying

Special equipment supplied by Greiner Bio-One enables the parallel washing of up to four Pelv**oCheck**® CT/NG chips at once. The additional equipment required for processing the Pelv**oCheck**® CT/NG chips is comprised of 2 oCheck® Washboxes and a handle for the magnetic slideholder of the Hybridisation Chamber.

The different working steps are shown in Figure 9.

- Carefully remove the magnetic slideholder containing the hybridised slides from the Hybridisation Chamber.
- Drop the slideholder containing the slides directly into the oCheck® Washbox containing washing solution I. Ensure that the magnetic side is facing up.
- Attach the oCheck® handle to the slideholder and begin the first of 2 washing steps.
- Wash the chip at room temperature (18 to 25 °C) in washing solution I by moving it quickly up and down for 20 seconds. The arrays must stay covered with washing solution at all times.
- Wash the chip for 30 seconds in washing solution II at 50 ± 2 °C by vigorously moving the slide holder up and down.

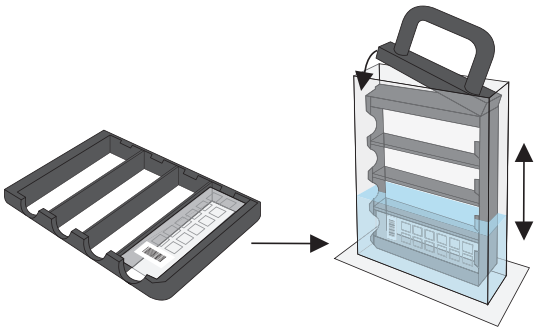
! Avoid the chip surface to run dry during the washing procedure!

Immediately, remove any liquid from the chip surface by centrifugation. If a special microcentrifuge for microarrays is used, centrifuge for 1 minute. If a centrifuge applicable for 50 mL tubes is used, place every washed Pelv**oCheck**® CT/NG chip into a separate 50 mL tube and centrifuge at room temperature (18 to 25 °C) for 3 minutes at 500 x g.

The Pelv**oCheck**® CT/NG chip is now ready for scanning and should be scanned immediately. For cleaning of the oCheck® Washboxes, rinse several times with water after each completed washing and drying procedure.

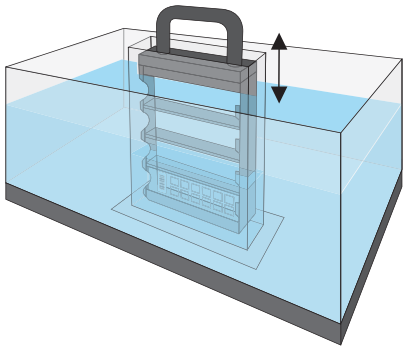
First washing step

- Carefully remove the magnetic slideholder from the Hybridisation Chamber.
- Quickly drop the slideholder into the oCheck® Washbox with washing solution I.
- Attach the oCheck® handle.
- Wash the Pelv**oCheck**® CT/NG chip(s) in washing solution I at room temperature (18 to 25 °C) for 20 seconds by moving the slideholder up and down.



Second washing step

- Wash the Pelv**oCheck**® CT/NG chip(s) in washing solution II in a water bath of 50 +/-2 °C for 30 seconds by moving the slideholder up and down.



Drying

- Immediately remove any liquid from the surface of the Pelv**oCheck**® CT/NG chips by centrifugation

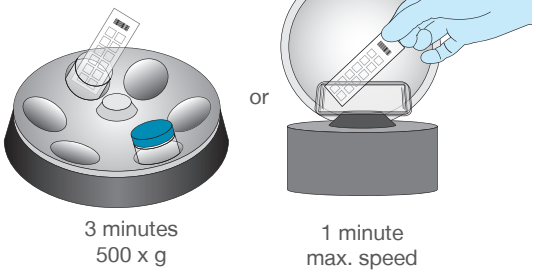


Figure 8: Working steps of the washing procedure

Different washing steps and drying procedure prior to the analysis of the Pelv**oCheck**® CT/NG chip with the CheckScanner™ and the CheckReport™Software.

8.6 Scanning and evaluation of the PelvoCheck® CT/NG chip

Place the Pelv**oCheck**® CT/NG chip(s) in the CheckScanner and proceed with scanning as described in detail in the User Guide of the CheckReport™Software.

When analysing pooled samples you have to document the ID numbers of each single sample in the "Comment" field. Alternatively, the Administrator of your system may add a specific sample annotation field for the documentation of the ID numbers of a sample pool via CheckReport™Admin tool (see CheckReport™Software User Guide).
For more detailed information about the installation of the CheckScanner™ and the CheckReport™Software, as well as computer system requirements, please consult the corresponding Instructions For Use of the CheckScanner™ and the CheckReport™Software.

Whenever analysing data using the CheckReport™Software, ensure that the version of the CheckReport™Software installed on your computer matches the version indicated on the currently used Pelv**oCheck**® CT/NG test kit. If the versions do not match, update the CheckReport™Software.

The latest Software version can be downloaded from the Greiner Bio-One website:
https://www.gbo.com/en_INT/know-how-services/download-center.html

9. TROUBLESHOOTING

For the troubleshooting refer to the respective Instructions for Use, e.g. oCheck® DNA Extraction Kit - CheckExtractor™, Pelv**oCheck**® CT/NG for manual handling or CheckExtractor™

10. TECHNICAL ASSISTANCE

If you have any questions, experiences or difficulties concerning oCheck® products, please do not hesitate to contact your local Greiner Bio-One distributor or the technical support department **(support.dx@gbo.com)**, staffed with experienced scientists with extensive practical and theoretical expertise in molecular biology and on oCheck® products.

11. PERFORMANCE CHARACTERISTICS OF PELVOCHECK® CT/NG

11.1 Analytical performance of PelvoCheck® CT/NG

11.1.1 Limit of Detection

The 95 % detection probability (DP95%) of the Pelv**oCheck**® CT/NG assay was determined with quantified stocks from a certified supplier (DSMZ). The DP95% was calculated for individual samples (stabilised human urine and swab equivalents) by applying Probit-analysis using the program SPSS (Version 11.0).

The Limit of Detection using Pelv**oCheck**® CT/NG (REF 504 288) in combination with CheckExtractor™ and the oCheck® DNA Extractor Kit-Pelv**oCheck**® has been confirmed.

Table 6: Pelv**oCheck**® CT/NG - Limit of Detection

Summary of the Limit of Detection of Pelv**oCheck**® CT/NG		
	Urine individual sample	**Swab individual sample**
CT	0.3 IFU/mL	0.4 IFU/mL
NG	0.9 CFU/mL	1.7 CFU/mL

*IFU/mL = Infectious Forming Unit per mL and CFU/mL = Colony Forming Unit per mL

11.1.2 Analytical specificity for CT and NG

The qualitative detection of decreasing numbers of CT or NG DNA genome copies was performed in presence of 10^6 copies/reaction of the opposite analyte. In a second experiment, the qualitative detection of 10 DNA genome copies of CT or NG per reaction was explored in the presence of increasing numbers of the opposite analyte. Pelv**oCheck**® CT/NG is able to differentiate between CT and NG and the specificity is 100 %. Pelv**oCheck**® CT/NG allows the detection of CT in presence of a 10,000-fold excess of NG genome copies per reaction and the detection of NG in presence of a 10,000-100,000-fold excess of CT genome copies per reaction.

11.1.3 Analytical specificity towards accompanying urogenital flora

The analytic specificity of the Pelv**oCheck**® CT/NG assay was tested in the presence of bacteria, fungi, viruses and human cells that may be commonly found or may be isolated from the human urogenital tract. Each isolate was analysed with the Pelv**oCheck**® CT/NG assay using at least 1 ng genomic DNA per test.

The following Non-CT and Non-NG organisms have been tested with Pelv**oCheck**® CT/NG. No positive signals were detected. Thus, the analytical specificity of the Pelv**oCheck**® CT/NG is 100 % towards common organisms of the human urogenital tract.

Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter lwoffii, Actinobacillus actinomycetemcomitans, Actinomyces odontolyticus, Actinomyces israelis, Bacillus subtilis, Bacteroides ureolyticus, Bacteroides uniformis, Bifidobacterium adolescentis, Bifidobacterium breve, Campylobacter concisus, Campylobacter gracilis, Campylobacter rectus, Candida albicans , Candida glabrata, Capnocytophaga gingivalis, Capnocytophaga ochracea, Capnocytophaga sputigena, Citrobacter amalonaticus, Citrobacter freundii, Citrobacter koseri, Citrobacter koseri , Chlamydophila psittaci, Chlamydophila pneumoniae, Clostridium difficile, Clostridium perfringens, Eikenella corrodens, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter sakazakii, Enterococcus durans, Enterococcus faecali, Enterococcus faecium, Escherichia coli, Eubacterium nodatum , Fusobacterium nucleatum, Gardnerella vaginalis , Hafnia alvei , Kingella denitrificans,

Klebsiella oxytoca, Klebsiella pneumoniae, Lactobacillus casei, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, Lactobacillus rhamnosus, Lactobacillus vaginalis, Mobiluncus muleris, Mogibacterium timidum, Morganella morganii, Mycoplama hominis, Moraxella osloensis, Mycoplasma hominis, Mycoplasma genitalium, Mycoplasma orale, Mycoplosma pneumoniae, Neisseria elongata, Neisseria meningitidis, Neisseria flavescence, Neisseria sicca, Neisseria subflava, Oigella urethralis, Peptoniphilus asaccharolyticus, Peptostreptococcus anaerobius, Peptostreptococcus micros, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Proteus hauseri, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida , Serratia marcescens, Staphylococcus aureus ssp. aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus, Stenotrophomonas maltophilia, Streptococcus agalactiae, Streptococcus constellatus, Streptococcus criceti, Streptococcus cristatus, Streptococcus gordonii, Streptococcus intermedius, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus parasanguinis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus sobrinus, Treponema denticola, Treponema pallidum, Ureaplasma uralyticum, Veillonella parvula, HPV-16.

11.2 Repeatability

For assessing test repeatability of Pelv**oCheck**® CT/NG, one technician analysed CT- and NG-spiked human urine samples (3-fold DP95%) and negative urine samples, as well as swab equivalents spiked with CT and NG (3-fold DP95%) and negative swab equivalents according to the standard protocol of Pelv**oCheck**® CT/NG (see Table 7). The samples were processed at one laboratory site and by using always the same equipment.

Table 7: Pelv**oCheck**® CT/NG - Repeatability

Target	Matrix	Concentration	Sample type	No. of positive analyses / No. of total analyses		No. of negative analyses / No. of total analyses CT/NG negative	Repeatability (%)
				CT positive	NG positive		
CT and NG	human urine	3-fold 95% DP	individual	122/122	122/122	6/6	100
CT and NG	swab equivalents		individual	122/122	122/122	6/6	100

For all urine and swab samples and each repeated analysis, the pathogens were detected and the analyses for the negative samples were negative. Thus, the repeatability of Pelv**oCheck**® CT/NG for urine and swab samples using Pelv**oCheck**® CT/NG (REF 504 288) in combination with CheckExtractor™ and the oCheck® DNA Extractor Kit-CheckExtractor™ has been confirmed, and is 100 % with 3-fold DP95% samples and negative controls resulting in an error probability of 0 %.

11.3 Reproducibility

The reproducibility for the Pelv**oCheck**® CT/NG test kit (REF 504 002) was determined using 128 clinical samples consisting of CT-positive, NG-positive and CT and NG negative urine samples, cervical and vaginal swabs. In case of urine samples, individual urine samples were spiked with reference materials for the generation of double-positive samples. The determination was carried out using the same method and kit components, with identical sample material, by different personnel and in different laboratories with different instrumentation. Results were rated concordant for positive samples, if identical pathogens were detected and for negative samples, if the analyses were valid but negative for the pathogens. For all concordant clinical samples, the results showed a 100 % positive and negative agreement.

For Pelv**oCheck**® CT/NG test kit (REF 504 288) using the CheckExtractor™ in combination with the oCheck® DNA Extraction Kit - CheckExtractor™, 92 of 92 urine samples showed concordant results. The performance agreement for urine samples is 100.0 %. 93 swab samples including cervical and vaginal swabs showed concordant results for analyses using the CheckExtractor™ in combination with the oCheck® DNA Extraction Kit - CheckExtractor™. The performance agreement for swab samples is 100.0 %.

11.4 Robustness

In order to evaluate the robustness of the Pelv**oCheck**® CT/NG test system variations of the following parameters were considered:

- Amount of polymerase
- Ramping mode of PCR cycler
- Variation of device type
- Amount of PCR product in hybridisation
- Volume of hybridisation buffer
- Hybridisation temperature
- Hybridisation time
- Washing temperature
- Washing time

The verified ranges of parameter values in which a robust detection of CT and NG is possible with Pelv**oCheck**® CT/NG are summarised in Table 8.

Table 8: Pelv**oCheck**® CT/NG robustness

Parameter	Range
Amount of polymerase	0.5-2 Units/rxn
Ramping mode	9,600 and Max
Device type	ABI GenAmp/GenAmp ABI Verity/Verity ABI GenAmp/ABI Verity
Amount of PCR product in hybridisation	10 µL ± 5 µL
Volume of hybridisation buffer	30 µL ± 5 µL
Hybridisation temperature	18°-28 °C
Hybridisation time	30 minutes ± 15 minutes
Washing temperature of the 50 °C wash step	50 °C ± 5 °C
Washing time	1st washing step: 20 seconds ± 10 seconds
	2nd washing step: 30 seconds ± 10 seconds

In case of robustness amplification, the presence of some bacteria common in the urogenital tract in excess can lead to amplification competition. In analysis of 111 clinical samples screening for Bacteroides ureolyticus, Moraxella osloensis, Neisseria meningitides Oigella urethralis, and Treponema pallidum, no critical effects, which could have influenced the qualitative result of Pelv**oCheck**® CT/NG analysis, were observed.

Additionally, carryover likelihood was investigated by hybridising highly CT/NG positive samples and negative controls alternately in a chess board arrangement on the Pelv**oCheck**® CT/NG chips. No carryover effects were observed.

11.5 Clinical Performance of PelvoCheck® CT/NG

Clinical performance characteristics of the Pelv**oCheck**® CT/NG assay in terms of positive and negative agreement with other NAAT (nucleic acid amplification technique)-based assays were established in two clinical studies.

The first clinical study evaluated urine samples collected with the recommended sampling system (Pelv**oCheck**® Collection Kit SAFE, Abbott multi-Collect Sample Collection Kit or native urine) of 1,649 young women between 18-25 years attending a clinical practice or a gynaecological day clinic for routine check-up (representing the typical CT-screening population). In addition, 115 pre-screened NG-positive or -negative samples from 18-25 years old women provided by a clinical laboratory were tested. The 1,649 urine samples included 50 CT-spiked samples in different concentrations mimicking the distribution of naturally infected women for lack of CT-positive samples. Sample preparation was carried out for Pelv**oCheck**® CT/NG with the oCheck® DNA Extraction Kit or the oCheck® DNA Extraction Kit - CheckExtractor™, for Roche with the MagNA Pure System and for Abbott RealTime CT/NG with the m2000 RealTime machine according to the manufacturer's instructions. Pelv**oCheck**® CT/NG analysis were compared with the commercially-available NAAT-based assays (Roche Cobas® TaqMan® CT assay) and a highly sensitive NG in-house PCR (*porA*, pseudo gene) of the clinical partner (see Table 8, 9). A total of 133 samples were analysed with a second reference method, Abbott RealTi*me* CT/NG (see Table 9, 10).

Table 9: Degree of the diagnostic performance of Pelv**oCheck**® CT/NG compared to the reference methods for CT.

Reference method	Quality criterion	Sample type	Agreement [%]	Agreement in sample number
Roche Cobas® TaqMan® CT	Positive agreement	urine / individual	98.8	80/81
	Negative agreement	urine / individual	99.9	1566/1568
	Total agreement	urine / individual	99.8	1646/1649
Abbott RealTi*me* CT/NG	Positive agreement	urine / individual	98.8	81/82
	Negative agreement	urine / individual	100.0	51/51
	Total agreement	urine / individual	99.2	132/133

Table 10: Degree of the diagnostic performance of Pelv**oCheck**® CT/NG compared to the reference methods for NG.

Reference method	Quality criterion	Sample type	Agreement [%]	Agreement in sample number
NG in-house PCR (*porA* gene)	Positive agreement	urine / individual	98.2	54/55
	Negative agreement	urine / individual	95.0	57/60
	Total agreement	urine / individual	96.5	111/115
Abbott RealTi*me* CT/NG	Positive agreement	urine / individual	98.2	56/57
	Negative agreement	urine / individual	98.3	57/58
	Total agreement	urine / individual	98.3	113/115

The comparison of the results of Pelv**oCheck**® CT/NG and Roche COBAS® TaqMan® CT as well as of Pelv**oCheck**® CT/NG and Abbott RealTi*me* CT/NG demonstrate a high number of concordant results in 1646/1649 samples (99.8 %), and 132/133 samples (99.2 %), respectively. Regarding Abbott RealTi*me* CT/NG as superior reference test, no false CT-positive results were obtained with both the Pelv**oCheck**® CT/NG test and the Roche COBAS® TaqMan® CT test (specificity 100 % for each test). The sensitivity of the Pelv**oCheck**® CT/NG test for CT DNA appears slightly higher than for Roche COBAS® TaqMan® CT: 82/83 (98.8 %) compared to 81/83 (97.6 %).

Generally, the total agreement of results obtained with the Pelv**oCheck**® CT/NG and the in-house porA PCR (111/115, 96.5 %) as well as of results derived from analyses with Pelv**oCheck**® CT/NG and Abbott RealTi*me* CT/NG (113/115, 98.2 %) was high. The sensitivity of the Pelv**oCheck**® CT/NG test for NG DNA was slightly higher (56/57) than of the in-house *porA* PCR (55/57) (98.2 % vs. 96.5 %), presumably due to a higher analytical sensitivity and possibly due to porA sequence variations recently described for a gonococcal strain isolated in Australia (Whiley et al. 2011, Eurosurveillance). The specificity of the Pelv**oCheck**® CT/NG (57/58) for NG DNA appears lower than of the in-house *porA* PCR (58/58) (98.3 % vs 100 %). This lower specificity, however, only depends on one positive result in one sample that was negatively tested by both the in-house *porA* PCR and the Abbott RealTi*me* CT/NG. The sample may contain NG DNA only detectable with the Pelv**oCheck**® CT/NG test, if it has the highest sensitivity of all assays used. In this context, a recent statement indicates that the confirmation of positive NAATs might be difficult if the confirmatory test has lower sensitivity than the initially used test. This is evident, when the sample is borderline positive and contains target sequences only in low copy numbers (Schachter et al. 2006, J Clin Microbiol). For this reason, the positive NAAT results that were not confirmed by another NAAT are not inevitably false positives, but may also represent false negative results of the confirmatory test.

The **second clinical study** evaluated cervical and vaginal swabs collected with the recommended sampling system of 434 women older than 18 years attending an ambulatory specialised for sexually transmitted diseases. Two sets of cervical and vaginal swabs were taken: the first set was collected with the respective sampling kits for cervical and vaginal swabs recommended for the analysis with the reference system Hologic/Gen-Probe APTIMA Combo 2® (APTIMA® Unisex Sample Collection Kit and APTIMA® Vaginal Swab Sample Collection Kit). The second set of cervical and vaginal swabs were sampled with the Pelv**oCheck**® Swab Collection Kit and analysed with Pelv**oCheck**® CT/NG and Abbott RealTi*me* CT/NG.
For diagnostic reason, all cervical and vaginal swabs collected with the suggested Hologic/Gen-Probe sampling kits were tested with the Hologic/Gen-Probe APTIMA Combo 2® for CT/NG in combination with the Hologic/Gen-Probe Panther machine.
Subsequently, all of the pre-screened cervical and vaginal swabs positive for CT and/or NG and a subset of the pre-screened cervical and vaginal swabs negative for CT and NG were analysed with Pelv**oCheck**® CT/NG and Abbott RealTi*me* CT/NG by using the second set of swabs sampled with the Pelv**oCheck**® Swab Collection Kit. Sample preparation was carried out for Pelv**oCheck**® CT/NG with the oCheck® DNA Extraction Kit or oCheck® DNA Extraction Kit - CheckExtractor™ and for Abbott RealTi*me* CT/NG with the m2000 RealTime machine according to the manufacturer's instructions.
The application of the Pelv**oCheck**® Swab Collection Kit for swab analysis in combination with the Abbott RealTi*me* CT/NG was tested prior to the clinical performance evaluation study. All tested samples showed the same performance irrespective of the used sampling kit (Pelv**oCheck**® Swab Collection Kit or the Abbott multi-Collect Sample Collection Kit).
Due to a low CT and NG prevalence and a poor attendance of women complying with the defined criteria, individual cervical and vaginal swabs tested negative for CT and NG with Hologic/Gen-Probe APTIMA Combo 2® for CT/NG were enriched with CT and NG reference material mimicking the natural distribution in cervical and vaginal swabs. In case of CT, 25 cervical and 25 vaginal swabs, and in case of NG, 46 cervical and 44 vaginal swabs were prepared and analysed with all three methods.

The results obtained with Pelv**oCheck**® CT/NG were compared to those of the Hologic/Gen-Probe APTIMA Combo 2® for CT/NG and Abbott RealTi*me* CT/NG. The positive and negative agreement as well as the total agreement were calculated (see Tables 11-13).

Table 11: Degree of the diagnostic performance of PelvoCheck® CT/NG compared to the reference methods for CT.

Reference method	Quality criterion	Sample type	Agreement [%]	Agreement in sample number
Hologic/Gen-Probe APTIMA Combo 2® for CT/NG (1st set of swabs)	Positive agreement	cervical	96.15	50/52
		vaginal	94.44	51/54
	Negative agreement	cervical	100.00	102/102
		vaginal	100.00	97/97
Abbott RealTi*me* CT/NG (2nd set of swabs)	Positive agreement	cervical	98.04	50/51
		vaginal	98.08	51/52
	Negative agreement	cervical	100.00	103/103
		vaginal	100.00	99/99

Table 12: Degree of the diagnostic performance of PelvoCheck® CT/NG compared to the reference methods for NG.

Reference method	Quality criterion	Sample type	Agreement [%]	Agreement in sample number
Hologic/Gen-Probe APTIMA Combo 2® for CT/NG (1st set of swabs)	Positive agreement	cervical	100.00	50/50
		vaginal	100.00	49/49
	Negative agreement	cervical	100.00	104/104
		vaginal	100.00	102/102
Abbott RealTi*me* CT/NG (2nd set of swabs)	Positive agreement	cervical	98.04	50/51
		vaginal	100.00	49/49
	Negative agreement	cervical	100.00	103/103
		vaginal	100.00	102/102

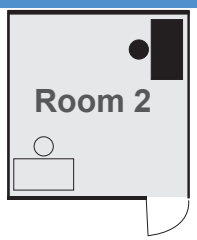
Table 13: Total concordance of results obtained with PelvoCheck® CT/NG and reference methods for all samples.

	Hologic/Gen-Probe APTIMA Combo 2® for CT/NG x PelvoCheck® CT/NG		Abbott RealTi*me* CT/NG x PelvoCheck® CT/NG		Hologic/Gen-Probe APTIMA Combo 2® for CT/NG x Abbott RealTi*me* CT/NG	
	cervical	vaginal	cervical	vaginal	cervical	vaginal
CT	98.70 %	98.01 %	99.35 %	99.34 %	99.35 %	98.68 %
NG	100.00 %	100.00 %	99.35 %	100.00 %	99.35 %	100.00 %

In general, the total agreement of the results obtained with all three methods was high regardless of the pathogen and the type of swab. The total agreement of Pelv**oCheck**® CT/NG with the results gained with both reference methods for CT in cervical swabs was 98.70-99.35 % and for vaginal swabs 98.01-99.34 %. In case of NG, the total agreement of Pelv**oCheck**® CT/NG with the results achieved with both reference methods was in cervical swabs 99.35-100 % and for vaginal swabs 100 %. The positive agreement of Pelv**oCheck**® CT/NG with the results obtained with both reference methods for CT for cervical swabs was 96.15-98.04 % and for vaginal swabs 94.44-98.08 %. In case of NG, the total agreement of Pelv**oCheck**® CT/NG with the results gained with both reference methods was for cervical swabs 98.04-100.00 % and for vaginal swabs 100.00 %.

Discrepant results between the Hologic/Gen-Probe APTIMA Combo 2® for CT/NG assay and the Pelv**oCheck**® CT/NG or Abbott RealTi*me* CT/NG assay, might be caused by the fact that two different sets of swabs had to be used, of which the APTIMA-swab was always taken first. In addition, most of the discrepant results showed alternating positive and negative results. In contrast, all cervical and vaginal swabs spiked with CT or NG (verifiable true positive swabs and equal for all 3 methods) were

tested positive and no discordant results compared to the two reference methods were detected. In no analysis, false positive CT or NG results were noticed with Pelv**oCheck**® CT/NG and therefore, the negative agreement of Pelv**oCheck**® CT/NG was 100 %.

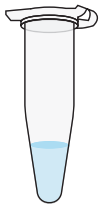


12. PELVOCHECK® CT/NG SHORT PROTOCOL

12.1 Room 2: Manual PCR-setup of reaction mix

! Preferably prepare dilution of Uracil-N-DNA Glycosylase and PelvoCheck® CT/NG PCR MasterMix in a clean bench.

- Dilute the Uracil-N-Glycosylase **1:50** in PCR-grade water
- Mix the UNG dilution carefully

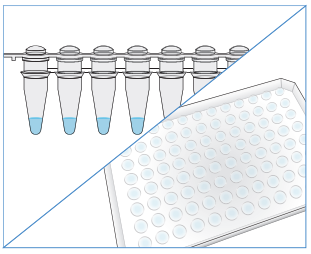


- Prepare the reaction mix for the required quantity of PCR reactions

	48 reactions
PelvoCheck® CT/NG PCR MasterMix	1154.2 µL
HotStarTaq® DNA Polymerase (5 U/µL)	5.8 µL
Uracil-N-Glycosylase (Dilution of 1:50, 0.02 U/µL)	58 µL
Total volume	1218 µL

- Mix the reaction mix carefully

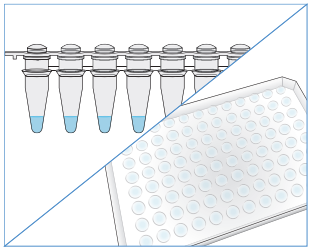
- Aliquot the reaction mix: add **21 µl** of the reaction mix for each PCR reaction into 0.2 mL PCR reaction tubes or a 96-well PCR plate.



12.2 Room 2: Manual PCR setup

OPTIONAL:

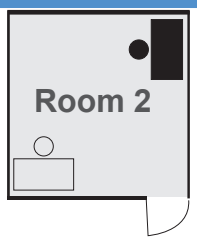
- Add 5 µl of DNA template for each PCR reaction.
- Mix thoroughly.



- If a PCR plate was used, seal the plate using Pierce Seal and Heatsealer. Use 170 °C as sealing temperatur and a duration of t2.0 seconds.

- Start the PCR reaction with the prepared thermal cycler program.

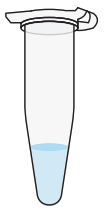
Time	Temp. °C	No. of cycles
20 min	37 °C	1
15 min	95 °C	1
30 s 60 s 30 s	94 °C 65 °C 72 °C	45
10 min	72 °C	1
Hold	10 °C	∞



12.3 Room 2: Automated PCR setup of reaction mix

! Preferably prepare dilution of Uracil-N-DNA Glycosylase and PelvoCheck® CT/NG PCR MasterMix in a clean bench.

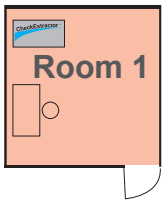
- Dilute the Uracil-N-Glycosylase 1:50 in PCR-grade water
- Mix the UNG dilution carefully



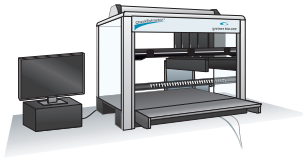
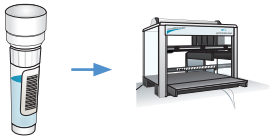
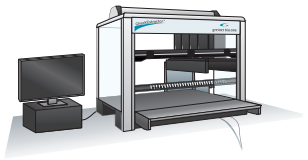
- Prepare the final MasterMix for the required quantity of PCR reactions

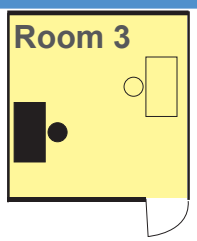
	48 reactions
PelvoCheck® CT/NG PCR MasterMix	1154.2 µL
HotStar Taq® DNA Polymerase (5 U/µL)	5.8 µl
Uracil-N-Glycosylase (Dilution of 1:50, 0.02 U/µL)	58 µl
Total volume before addition of sample DNA	1218 µl

- Mix the final MasterMix carefully



12.4 Room 1: Automated PCR setup using CheckExtractor™

➡ Start PCR Setup method. ➡ Complete run information. ➡ Load tip carrier with sufficient amount of tips. ➡ Load plate carrier with elution plate and empty PCR plate.	
➡ Load the barcoded MasterMix preparation tube onto the tube carrier. ➡ Load the tube carrier. ➡ Start PCR setup run.	
➡ After the PCR setup run has finished, seal the PCR plate (refer to chapter 8.4).	
➡ Transfer the sealed plate to the PCR cycler and start the thermal cycler program.	
➡ After the PCR setup run has finished, start unloading and close the elution plate. ➡ Start the cleaning procedure.	



12.5 Room 3: Hybridisation - Preparation / Hybridisation reaction

!

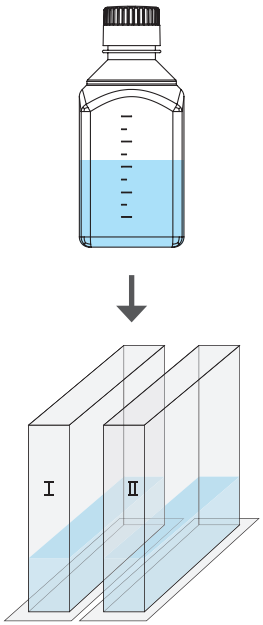
Begin preparations at least 30 minutes prior to hybridisation.

- Equilibration of hybridisation and washing buffers
- Preparation of hybridisation chamber
- Pre-incubation of chips
- Preheating of water bath to 50 °C
- Preparation of washing solutions

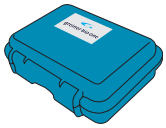
- Dissolve potential precipitates in the hybridisation and washing buffers by equilibration to room temperature (18-25 °C) and mix well
- Prepare the washing solution mix for the amount of Pelv**oCheck**® CT/NG chips to be analysed.

Components	Number of PelvoCheck® CT/NG chips
	4
Purified water	400 mL
PelvoCheck® CT/NG Buffer A	40 mL
PelvoCheck® CT/NG Buffer B	5 mL
Total volume	445 mL

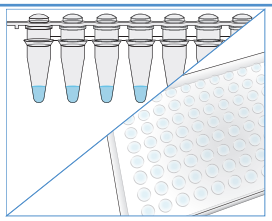
- Aliquot the washing solution mix into two oCheck® WashBoxes
- Preheat washing solution II in a water bath at **50 °C**



- Prepare the Hybridisation Chamber
- Pre-incubate the amount of Pelv**oCheck**® CT/NG chips to be analysed in the prepared Hybridisation Chamber at room temperature (18-25 °C)

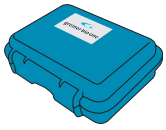


- Mix PCR products and briefly spin down
- Mix Hybridisation Buffer and briefly spin down
- Mix 30 µl Pelv**oCheck**® CT/NG Hybridisation Buffer with 10 µl PCR product in a 0.2 ml PCR reaction tube of a PCR strip
- Mix thoroughly and briefly spin down

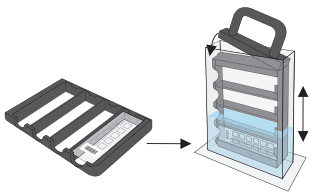
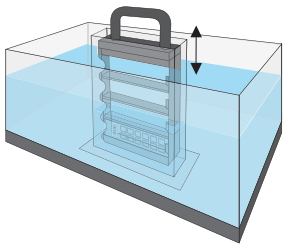
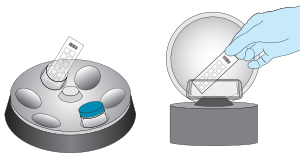


- Transfer 30 µl of the hybridisation mix into each well of the Pelv**oCheck**® CT/NG chip using a multichannel pipette
- Avoid air bubble formation

- Incubate the Pelv**oCheck**® CT/NG chip for exactly 30 minutes at room temperature (18-25 °C)



12.6 Room 3: Washing and drying / Scanning and evaluation

➡ Remove the magnetic slideholder from the Hybridisation Chamber ➡ Drop the slideholder into the oCheck® Washbox with washing solution I ➡ Attach the oCheck® Handle ➡ Wash the Pelv**oCheck**® CT/NG chip(s) in washing solution I at room temperature (18-25 °C) for 20 seconds	
➡ Wash the Pelv**oCheck**® CT/NG chip(s) in preheated washing solution II in a water bath at 50 °C for 30 seconds	
➡ Remove any liquid from the Pelv**oCheck**® CT/NG chip surface by centrifugation	 3 minutes 500 g or 1 minute max. speed
➡ Scan the Pelv**oCheck**® CT/NG chip(s) with the CheckScanner™ ➡ Perform scanning and analysis as described in the User Guide of the CheckReport™Software ➡ Create reports