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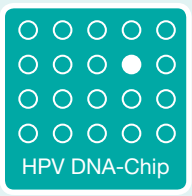

PapilloCheck®
PapilloCheck® high-risk

Instructions For Use

Diagnostic kit for the genotyping of human papillomavirus types 6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44/55, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 (Papillo**Check**®) or types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (Papillo**Check**® high-risk) in cervical specimens

For in-vitro diagnostic use
by professional laboratory personnel only

Revision: BQ-013-10 / October 2017

Greiner Bio-One GmbH
Maybachstr. 2
72636 Frickenhausen
Germany
Made in Austria

REF 465 060
505 060

COPYRIGHT INFORMATION
Instructions For Use Papill**oCheck**® Test Kit

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Notice

Trademark right
The used brand names and labels are the property of the respective owner.

Important information for the user
For the correct use of the Papill**oCheck**® Test Kit, 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
Papill**oCheck**® is a diagnostic kit and intended to be used for the qualitative detection and genotyping of 24 types of the human papillomavirus in DNA preparations from human cervical smears. The kit is intended to be used by qualified personnel only.

Papill**oCheck**® high-risk is a diagnostic kit and intended to be used for the qualitative detection and genotyping of 14 types human genital high-risk papillomaviruses (hrHPV) in DNA preparations from human cervical smears. The kit is intended to be used by qualified personnel only.

Papill**oCheck**® 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 Papill**oCheck**® should be interpreted in combination with other clinical or laboratory findings.

The Papill**oCheck**® Test Kits are for laboratory use only, not for drug, household, or other purposes. These products are intended for professional users only, such as technicians or physicians trained in molecular biology techniques.

Unless otherwise indicated, in this Instructions For Use the name Papill**oCheck**® is used for both variants, the Papill**oCheck**® and the Papill**oCheck**® high-risk.

GLOSSARY OF SYMBOLS

										
en	Batch code	Do not reuse	Use by date	Consult Instruc-tions for Use	Cata-logue Number	Manufac-turer	Not to be used in case package is dama-ged	Tempera-ture limit	Contents sufficient for <n> tests	GHS pictograms with signal words DANGER or WARNING

				
en	CE marks	In vitro di-agnostic medical device	Store in the dark	Important Note

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1. KIT CONTENT

PapilloCheck® and PapilloCheck® high-risk Test Kit[1]	Content	Quantity
PapilloCheck® (REF 465 060)		
PapilloCheck® PCR MasterMix	1 x PapilloCheck® PCR MasterMix[2]	1 x 1200 µL
PapilloCheck® Slidebox, 4 x 12 Arrays	1 x PapilloCheck® Slidebox, with 4 PapilloCheck® chips[3] à 12 arrays, for 48 samples	1
PapilloCheck® Hyb.-Buffer	2 x PapilloCheck® Hybridisation Buffer	2 x 1,000 µL
PapilloCheck® BUF A conc.	2 x PapilloCheck® Wash buffer A, concentrate	2 x 40 mL
PapilloCheck® BUF B conc.	1 x PapilloCheck® Wash buffer B, concentrate	1 x 15 mL
UNG-Dilution Preparation Tube	PCR-grade water	1 x 995 µL
MasterMix Preparation Tube (usage only in combination with automation)	empty	1
Flyer	Download Instructions For Use	1
PapilloCheck® high-risk (REF 505 060)		
PapilloCheck® high-risk PCR Master-Mix	1 x PapilloCheck® high-risk PCR MasterMix[2]	1 x 1200 µL
PapilloCheck® high-risk Slidebox, 4 x 12 Arrays	1 x PapilloCheck® high-risk Slidebox, with 4 PapilloCheck® high-risk chips[3] à 12 arrays, for 48 samples	1
PapilloCheck® high-risk Hyb.-Buffer	2 x PapilloCheck® high-risk Hybridisation Buffer	2 x 1,000 µL
PapilloCheck® high-risk BUF A conc.	2 x PapilloCheck® high-risk Wash buffer A, concentrate	2 x 40 mL
PapilloCheck® high-risk BUF B conc.	1 x PapilloCheck® high-risk Wash buffer B, concentrate	1 x 15 mL
UNG-Dilution Preparation Tube	PCR-grade water	1 x 995 µL
MasterMix Preparation Tube (usage only in combination with automation)	empty	1
Flyer	Download Instructions For Use	1

[1] One PapilloCheck® or PapilloCheck® high-risk Test Kit is sufficient for the analysis of 48 specimens.
[2] Contains all components required for PCR except DNA Taq Polymerase and the Uracil-N-Glycosylase.
[3] One PapilloCheck® chip contains 12 PapilloCheck® microarrays. In this Instructions For Use the words chip(s) and slide(s) are used as synonyms.

2. CONSUMABLES, EQUIPMENT AND HARDWARE REQUIRED

PapillоCheck® 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
PapillоCheck® Test Kit	465 060	1 kit for 48 reactions
PapillоCheck® high-risk Test Kit	505 060	1 kit for 48 reactions
PapillоCheck® Collection Kit	465 075	1 kit for 50 samples
oCheck® DNA Extraction Kit / Single Column Preparation	515 040	1 kit for 50 preparation
Sterile, DNase-free micropipette filter tips[4]		
0.5 µL - 10 µL	765 288	96/960
0.5 µL - 20 µL	774 288	96/960
10 µL - 100µL	772 288	96/960
10µL - 200 µL	739 288	96/960
100 µL - 1000 µL	750 288	60/600
50 mL polypropylene tube[5]	210 261	25/450
1.5 mL reaction tube	616 201	500/4000
Plastic pipettes for pipettor		
Pipette 10 mL	607 180 or 607 160	1/200
Pipette 25 mL	760 180 or 760 160	1/200
Pipette 50 mL	786 180 or 768 160	1/200

[4] Some of the mentioned tip sizes are optional depending on the micropipettes available.
[5] Only required if a slide centrifuge is not available.

Consumables	Greiner Bio-One Cat. No.	Quantity
CheckScanner™	862 070	1
CheckReport™Software Basic	862 080	1
CheckReport™Software Papill**oCheck**® plugin	862 081	1
CheckReport™Software Papill**oCheck**® high-risk plugin	862 088	1
PC system including CheckReport™Software and Papill**oCheck**® or Papill**oCheck**® high-risk plugin	862 910	1
Starter Package CheckScanner™ (CheckScanner™, PC system, CheckReport™Software, Papill**oCheck**® or Papill**oCheck**® high-risk plugin)	862 170	1
oCheck® Hybridisation Chamber with slide holder	447 070	1
Magnetic handle for slide holder	447 001	1
oCheck® Washbox[6]	447 020	1

Enzymes required

- Taq Polymerase: HotStarTaq® DNA Polymerase 5 U/µl (Qiagen; 203203, 203205, 203207, 203209)
- Uracil-N-Glycosylase: Uracil-DNA Glycosylase 1 U/µL (Thermo Scientific; #EN0361, #EN0362)

Additional consumables required

- PCR-grade water
- Purified water
- Single-use gloves
- DNA Away™ (Thermo Scientific; #7008, #7009, #7010)
- Please only use appropriate and PCR-compliant 8-tube-strips, single tubes or plates recommended by the manufacturer of the cycler used.
- To optimise the workflow the use of 8-tube-strips is recommended.

Additional equipment required

- Microcentrifuge for 1.5 and 2 mL reaction tubes
- Centrifuge for 50 mL polypropylene tubes
- Microcentrifuge for single 0.2 mL reaction tubes or 8-tube PCR strips
- PCR thermal cycler:
  GeneAmp® PCR system 9700 (Applied Biosystems)
  Veriti™ 96-Well Thermal Cycler (Applied Biosystems)
  peqSTAR 96X Universal (PEQLAB Biotechnology GmbH)
- Water bath (50 °C)
- Micropipettes (different ranges from 1 - 1000 µL)
- 8-Channel multipipette (range: 5 - 50 µL), e.g. Brand Transferpette®-8 (Brand)
- 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 Papill**oCheck**® washing procedure three oCheck® Washboxes are required.

3. SHIPMENT AND STORAGE

The shipment of the Papill**oCheck**® Test Kit takes place at ambient temperature. 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 Papill**oCheck**® chips always in the closed zipper bag with desiccant. All components must be stored in the original kit packaging to avoid mixed batches. Stored correctly, the Papill**oCheck**® 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.

Storage of the Papill**oCheck**® Test Kit at 4 to 8 °C may result in precipitation of salts in the Hybridisation Buffer and Buffer B. Precipitates must be dissolved before use by equilibration to room temperature (18 to 25°C) and mixing and vortexing the tube/bottle.

Product	Storage
Papill**oCheck**® Test Kit	4 to 8 °C, protected from light
Papill**oCheck**® high-risk Test Kit	4 to 8 °C, protected from light

4. SAFETY INSTRUCTIONS

The Papill**oCheck**® Test Kit is for laboratory use only, not for drug, household, or other purposes. DNA chips must be used in a dust free environment. The deposition of dust and other particles on the slides may result in unsatisfactory or invalid results.

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

Regulatory Information:
The following components of the Papill**oCheck**® 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	
Papill**oCheck**® Hybridisation Buffer, guanidine thiocyanate, 20-60 %, 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. Supplemental hazard information (EU): Contact with acids liberates very toxic gas.
Papill**oCheck**® Buffer B, sodium dodecyl sulfate, < 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 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.

The current version of the Safety Data Sheet for this product can be downloaded from the Greiner Bio-One website:
www.gbo.com ➠ Know-How & Services ➠ Download-Center

5. WASTE DISPOSAL

After washing and drying of the Papill**oCheck**® chip, the washing solutions I and II can be discarded without any special precautions. Dispose the used Papill**oCheck**® 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

Persistent infection with a carcinogenic human papillomavirus (HPV) is found in virtually all cases of cervical cancer – the second most common cancer in women worldwide[7]. To date more than 100 HPV types have been identified, of which about 40 types are sexually transmitted and infect the genital mucosa. Cervical HPV types are classified into a high-risk (high-risk HPV, hrHPV) and a low-risk group (low-risk HPV, lrHPV). Whereas the high-risk HPV types are associated with an increased risk of developing cervical cancer, low-risk HPV types mainly cause benign genital warts[8]. However, even within the high-risk group, the relative risk for the development of cancer or cervical intraepithelial lesions (CIN) is dependent on the type[9]. About 70 % of all cervical cancer cases are linked to a persistent infection with either HPV 16 or 18. The most prevalent low-risk types are HPV 6 and 11. On the basis of the nearly absolute etiologic link between carcinogenic HPV and cervical cancer, testing for hrHPV is now being considered for primary cervical cancer screening[10].

6.2 HPV types detectable with PapilloCheck®

PapilloCheck® allows the identification of 18 high-risk ☐ and 6 low-risk ☐ types of the human papillomavirus (HPV) (Table 1), whereas the PapilloCheck® high-risk allows the identification of 14 high-risk ☐ types of the human papillomavirus (hrHPV) (Table 2).

Table 1: HPV types detectable with PapilloCheck®

HPV 16	HPV 45	HPV 59	HPV 6
HPV 18	HPV 51	HPV 66	HPV 11
HPV 31	HPV 52	HPV 68	HPV 40
HPV 33	HPV 53	HPV 70	HPV 42
HPV 35	HPV 56	HPV 73	HPV 43
HPV 39	HPV 58	HPV 82	HPV 44 / HPV 55*

* PapilloCheck® does not allow the differentiation between HPV 44 and HPV 55.

Table 2: hrHPV types detectable with PapilloCheck® high risk

HPV 16	HPV 51
HPV 18	HPV 52
HPV 31	HPV 56
HPV 33	HPV 58
HPV 35	HPV 59
HPV 39	HPV 66
HPV 45	HPV 68

[7] Walboomers, J. et al (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 189(1):12-9., Sexually Transmitted Infections 76:80-87

[8] Burd EM. Human papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16:1–17.

[9] Bosch F.X. et al. (2008). Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine. 26 Suppl 10:K1-16.

[10] Meijer, C.J. et al. (2009). Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 124(3):516-20.

6.3 Assay principle

Papill**oCheck**® is a microarray-based test kit for the detection and genotyping of a fragment of the E1 gene of the human papillomavirus (HPV) genome. The assay procedure is summarised in Figure 1.

Prior to the Papill**oCheck**® analysis, DNA must be extracted from a cervical smear specimen. Specimen collection and DNA extraction are not part of the Papill**oCheck**® Test Kit. Dedicated products for specimen collection (Papill**oCheck**® Collection Kit) and DNA extraction (oCheck® DNA Extraction Kit) are also available from Greiner Bio-One and must be separately purchased (see ordering information in chapter 2).

After the extraction of viral and human genomic DNA from a cervical specimen, a 350 bp fragment of the viral E1 gene is amplified by polymerase chain reaction (PCR) in the presence of a set of HPV-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 cervical specimen (sample control) and an internal control-template present in the Papill**oCheck**® PCR MasterMix is amplified to monitor the performance of the PCR (PCR control). In addition, the Papill**oCheck**® 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 chapter 8.2.2).

The PCR products are then hybridised to specific DNA probes and on-chip controls attached to the Papill**oCheck**® chip surface. Every chip contains 12 DNA-microarrays, allowing the simultaneous analysis of 12 cervical samples. During hybridisation, the bound DNA is fluorescently labelled and unbound DNA is removed in the subsequent washing steps. The hybridisation efficiency is monitored (hybridisation control).

Finally, the Papill**oCheck**® 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 HPV-specific amplification products as well as the controls (see chapter 6.4.2). The CheckReport™Software allows the visualisation, analysis and evaluation of the results and automatically shows the corresponding values of both the detected HPV types and the controls in a detailed report.

The report clearly indicates the presence or absence of one or more of the 24 HPV types or 14 hrHPV types detectable and the comprehensive on-chip controls render the analysis highly reliable.

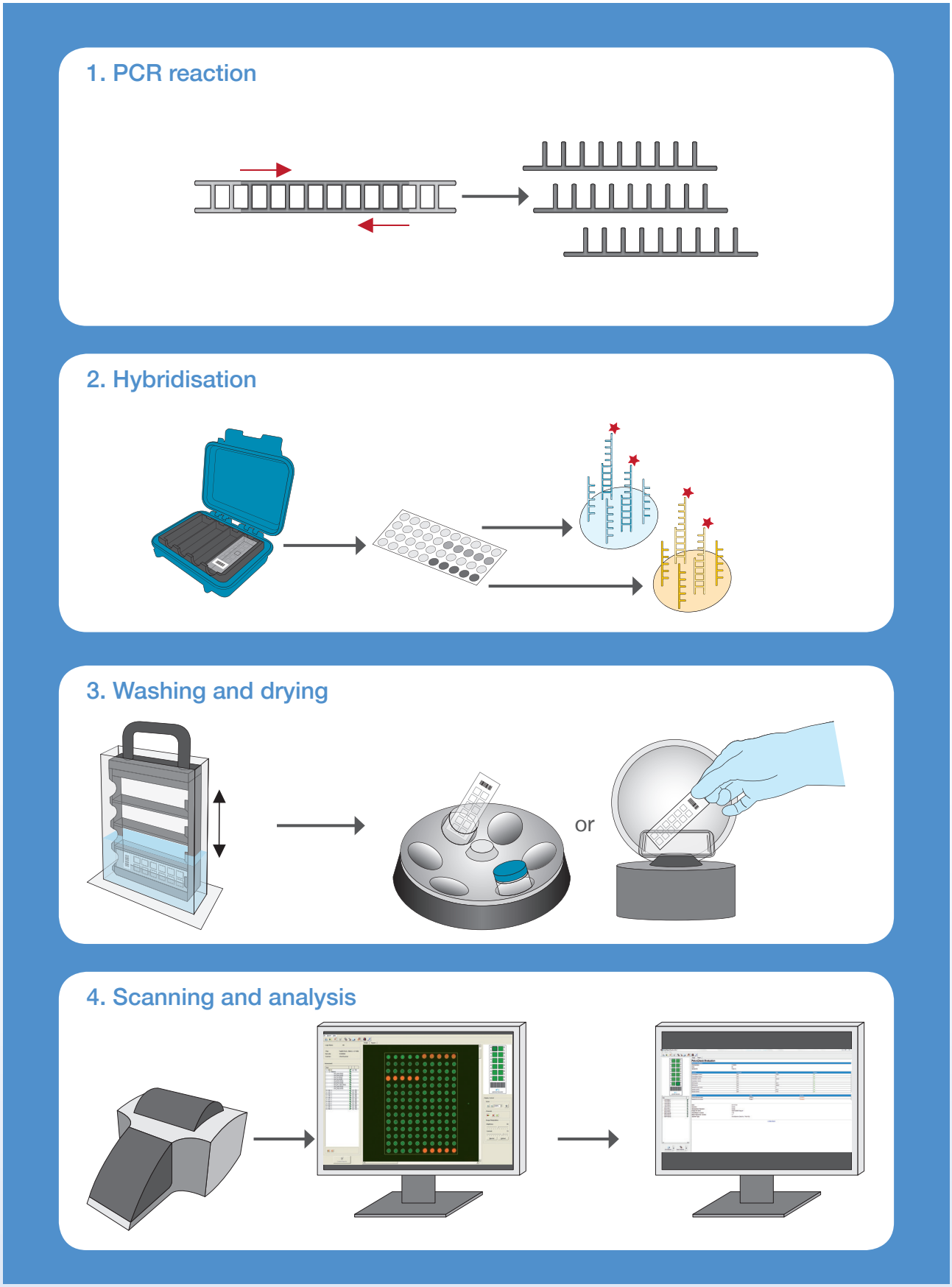


Figure 1: PapilloCheck® assay procedure

1. **PCR reaction:** After DNA extraction, a 350 bp fragment of the viral E1 gene and fragments of two control targets are amplified by PCR. The amplification products are then hybridised to complementary DNA probes on the chip.
2. **Hybridisation:** Each HPV type is detected by a specific DNA probe present in five replicates. During hybridisation the fluorescence labelling is introduced.
3. **Washing & drying:** Unbound DNA is removed in the subsequent washing steps.
4. **Scanning & analysis:** The PapilloCheck® chip is scanned, analysed and evaluated using the CheckScanner™ and CheckReport™Software. A report is created that clearly indicates the presence or absence of one or more of the HPV types detectable.

6.4 Design of the PapilloCheck® chip

6.4.1 PapilloCheck® chip layout

Each Papill**oCheck**® or Papill**oCheck**® high-rsik chip contains 12 microarrays designated as well A1 - B6. In each well a single microarray comprising different probes is spotted. Each single microarray is bordered by an elevated rim. Each probe is spotted in five replicates. The microarray layout is illustrated in Figure 2. The described on-chip controls are further explained in chapter 6.4.2.

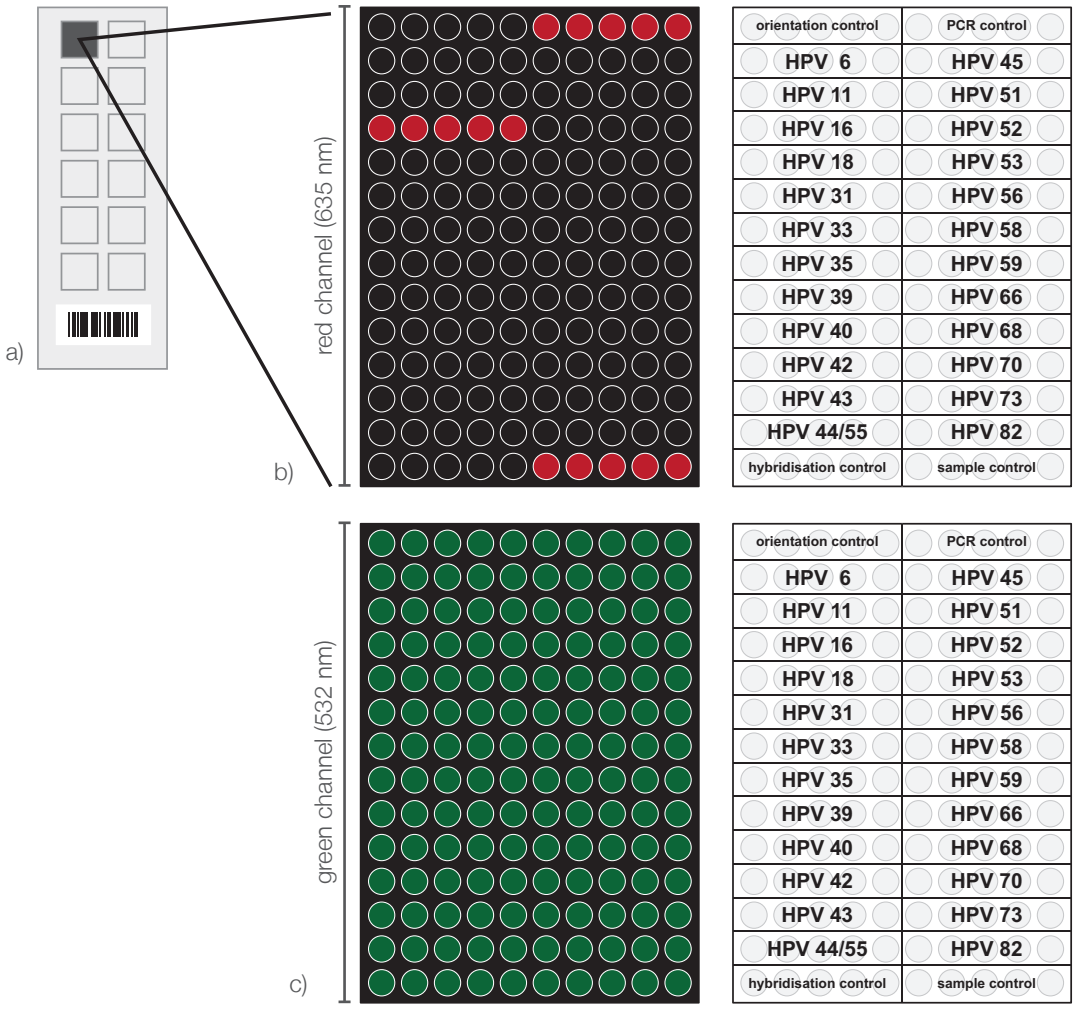


Figure 2: Design of the Papill**oCheck**® chip
a) Schematic drawing of the Papill**oCheck**® chip. b) and c) Images displayed by the CheckReport™Software for the two different excitation wavelengths used for scanning (b) red channel: 635 nm; c) green channel: 532 nm) and schematic drawings of the Papill**oCheck**® microarray layout. HPV type-specific probes and on-chip controls are indicated.

6.4.2 On-chip controls

The design of the Papill**oCheck**® DNA-chip incorporates comprehensive on-chip controls. Several control systems monitor all critical steps of both the assay and chip processing, including specimen quality and DNA extraction (sample control), quality 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 HPV types the CheckReport™Software automatically shows both the corresponding values of the controls and the detected HPV types in a detailed report. For read-out of the different controls, both excitation wavelengths of the CheckScanner™ are used. For the control of assay 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).

Sample control
Papill**oCheck**® monitors the quality of the specimen 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 extracted DNA from the cervical specimen, 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 specimen is positive for HPV, the analysis remains valid. In this case, the failed sample control is a direct result of competition between accompanying DNA and an excess of target DNA during PCR. If, on the other hand, the specimen is negative for HPV, 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).

PCR control
Papill**oCheck**® also monitors the performance of the PCR. Amplification of an internal control template present in the Papill**oCheck**® PCR MasterMix generates a signal on the PCR control spots on the Papill**oCheck**® 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).

If the amount of HPV 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 HPV-specific probe must exceed a predefined threshold in order for the test to be considered valid.

Hybridisation control
Papill**oCheck**® monitors the efficiency of the hybridisation through use of a fluorescence labelled probe within the Papill**oCheck**® Hybridisation Buffer, which hybridises to specific DNA sequences on the Papill**oCheck**® chip. An adequate hybridisation efficiency results in fluorescence signals on each array spot. The results of five hybridisation control spots on the Papill**oCheck**® chip are also assessed by the CheckReport™Software.

Orientation and printing control

The orientation control spots of the Papill**oCheck**® 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. The printing control on the Papill**oCheck**® chip monitors the presence and the hybridisation of the spots. 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 PAPILLOCHECK® WORKFLOW

The following description of the workflow is equal for application of the products Papill**oCheck**® and Papill**oCheck**® high-risk. Please follow the description for Papill**oCheck**® using only components of the Papill**oCheck**® Test Kit. Please follow the description for Papill**oCheck**® high-risk using only components of the Papill**oCheck**® high-risk Test Kit.

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.

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

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 setup 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.

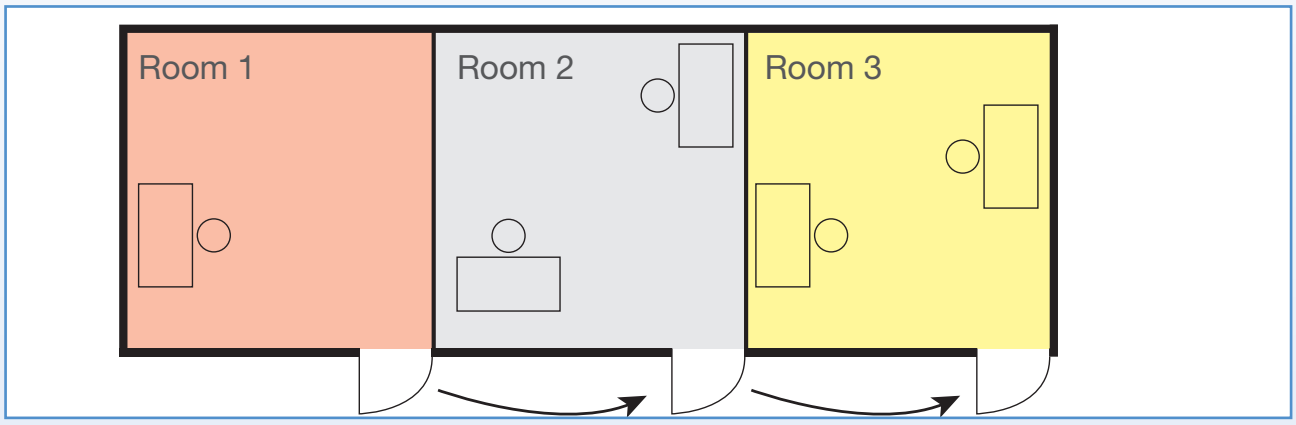


Figure 3: Room separation for manual processing

Room 1: The entire DNA extraction procedure must be performed in this room.
Room 2: Within this room, the reaction mix for the PCR is set up 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.
Room3: Within the third laboratory room the hybridisation reaction, washing steps and chip drying take place. Additionally, the CheckScanner™ in conjunction with the CheckReport™Software is used for the final analysis of the Papill**oCheck**® 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 Warnings and precautions

7.3.1 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™ (Thermo Scientific) 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.

7.3.2 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.

7.3.3 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 all equipment, e.g. PCR cyclers or pipettes.
- 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.

7.3.4 Working safely

- Always wear a suitable lab coat, disposable gloves and protective goggles!
- The kits are for in vitro diagnostic use only and should be exclusively used by personnel trained in in vitro diagnostic laboratory practice.
- 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 airflow conditions until sample lysis is completed. Handle and dispose 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.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling of samples and reagents.

The current version of the Material Safety Data Sheet for this product can be downloaded from the Greiner Bio-One website:
www.gbo.com ➠ Know-How & Services ➠ Download-Center

8. PAPILLOCHECK® 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 one or more of the HPV types detectable in each analysed cervical sample. Figure 4 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 ➠.

> **!** Specimen collection, DNA extraction and analysis with the CheckReport™Software are not part of the Papillo**Check**® 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 Papillo**Check**® Collection Kit, oCheck® DNA Extraction Kit and the CheckReport™Software.

8.1 Specimen collection and DNA extraction

8.1.1 Specimen collection

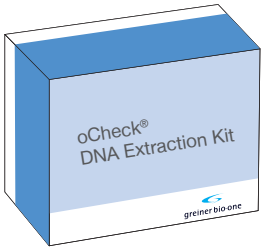
Specimen collection is not part of the Papill**oCheck**® Test Kit. A dedicated collection kit for cervical specimens (Papill**oCheck**® Collection Kit) is also available from Greiner Bio-One (see ordering information in chapter 2).

Papill**oCheck**® has been validated using DNA prepared with the oCheck® DNA Extraction Kit from human cervical smears collected with one of the following collection systems or media:

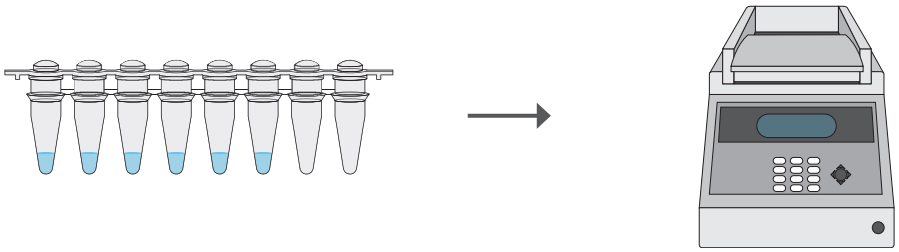
- Papill**oCheck**® Collection Kit (Greiner Bio-One, Frickenhausen, Germany)
- PreservCyt® (Hologic, Bedford, MA, USA)
- Surepath™ (BD, Franklin Lakes, NJ, USA)
- STM™ (Qiagen, Hilden, Germany)
- Easyfix (VWR, Radnor, PA, USA)
- Cyt-ALL (Alphapath, Mudaison, France)

For further information on suitable transport media or DNA extraction systems, please contact your local Greiner Bio-One distributor or consult the Greiner Bio-One website:
www.gbo.com ➠Know-How & Services ➠ Download-Center

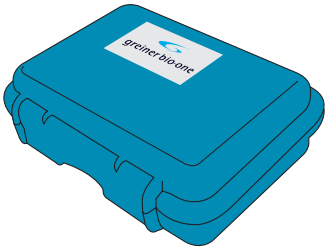
Chapter 8.1.2 DNA extraction



Chapter 8.2 PCR



Chapter 8.3.2 Hybridisation



Chapter 8.3.3 Washing and drying

Chapter 8.4 Scanning and evaluation

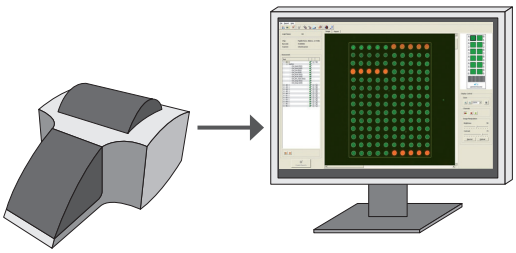


Figure 4: Overview of the different PapilloCheck® working steps

8.1.2 DNA Extraction

DNA extraction is not part of the Papill**oCheck**® Test Kit. Extraction of DNA prior to the Papill**oCheck**® analysis must be performed using the oCheck® DNA Extraction Kit, also provided by Greiner Bio-One (see chapter 2). Please follow the Instructions For Use carefully when using the oCheck® DNA Extraction Kit.

For DNA extraction with the oCheck® DNA Extraction Kit, 250 µL of sample solution must be used.

Human cervical smear samples which are collected
- with the Papill**oCheck**® Collection Kit (Greiner Bio-One, Frickenhausen, Germany)
- in PreservCyt® collection medium (Hologic, Bedford, MA, USA)
- in Easyfix (VWR, Radnor, PA, USA) or
- Cyt-ALL (Alphapath, Mudaison, France)

can be directly processed.

Cervical samples which are collected in
- Surepath™ collection medium (BD, Franklin Lakes, NJ, USA) must be washed before use: centrifuge 250 µL of the sample for 5 minutes at 11,000 x g and resuspend the pellet in 250 µL of distilled water. Subsequently, the 250 µL can be processed with the oCheck® DNA Extraction Kit.

Cervical samples which are collected in
- STM™ collection medium (Qiagen, Hilden, Germany) must be diluted: use 100 µL of the sample and add 150 µL of distilled water. The 250 µL of diluted sample can be processed with the oCheck® DNA Extraction Kit.

In general, if the cervical smear sample appears to be very concentrated and already aggregated, the sample must be diluted and homogenised before beginning with the DNA extraction!

Cervical smear samples which are very dilute and with no visible cells should be concentrated in order to achieve a higher cell yield for DNA extraction: centrifuge up to 1000 µL of the sample for 5 minutes at 11,000 x g and resuspend the pellet in 250 µL of distilled water. Subsequently, the 250 µL can be processed with the oCheck® DNA Extraction Kit.

!

This concentration step is only suitable for cervical smear samples which were collected with the Papill**oCheck**® Collection Kit, in PreservCyt®, Easyfix and Cyt-All collection medium or in Surepath™ collection medium. If the STM™ collection medium was used, a concentration by centrifugation is not possible.

8.2 Polymerase chain reaction (PCR)

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® Polymerase and Uracil-N-Glycosylase are required but not provided with the Papill**oCheck**® Test Kit and must be separately purchased (see chapter 2).

!

The Papill**oCheck**® Test Kit has been validated using HotStarTaq® Polymerase from Qiagen and Uracil-N-Glycosylase from Thermo Scientific (see chapter 2). It is mandatory to use these enzymes in order to achieve the claimed performance.

8.2.1 Thermal cycler setup

The Papill**oCheck**® Test Kit has been validated with the following thermal cyclers:

- GeneAmp® PCR system 9700 (Applied Biosystems)
- Veriti™ 96-Well Thermal Cycler (Applied Biosystems)
- peqSTAR 96X Universal (PEQLAB Biotechnologies GmbH).

> ! 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 Papill**oCheck**® PCR is summarised in Table 3.

Table 3: Thermal cycler program of the Papill**o**Check® PCR

Time	Temp. °C	No. of cycles
20 min	37 °C	1
15 min	95 °C	1
30 s 25 s 45 s	95 °C 55 °C 72 °C	40
30 s 45 s	95 °C 72 °C	15
Hold	10 °C	∞

In addition, the following run parameters must be set for each thermal cycler. For a description on how to set these parameters see the Instructions For Use of the respective thermal cycler.

GeneAmp® PCR system 9700 (Applied Biosystems)
Set the reaction volume to 26 µL, the ramp speed to "9600" and use lid temperature of 103 °C.

Veriti™ 96-Well Thermal Cycler (Applied Biosystems)
Use the Convert Method tool of the Veriti™ 96-Well Thermal Cycler to enter the Papill**oCheck**® PCR program and choose the "9600 Emulation Mode". Set the reaction volume to 26 µL and the temperature of the lid to 103 °C.

peqSTAR 96X Universal (PEQLAB Biotechnologies GmbH)
Use the preprogrammed Papill**oCheck**® PCR program "PapilloCheck.js" provided together with thermal cycler. Usually the programm can be opened under the following path: local/Scripts/GreinerBioOne/PapilloCheck.js.

8.2.2 Uracil-N-Glycosylase (UNG) treatment

The Papill**oCheck**® PCR MasterMix contains dUTP which is incorporated into the amplification products during the Papill**oCheck**® 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[11].

[11] Purchase of Papill**oCheck**® 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.

- Dilute the Uracil-N-Glycosylase 1:200 in PCR-grade water. For preparing this dilution of the Uracil-N-Glycosylase you can use the PCR-grade water from the UNG-Dilution Preparation Tube, that is included in the Papill**oCheck**® Test Kit. Use a fresh UNG dilution for each Papill**oCheck**® PCR reaction setup (see chapter 8.2.3). 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.

The original concentration of the Uracil-N-Glycosylase is 1 U/µL. Consequently the concentration of the dilution is 0.005 U/µL.

> **!** The Papill**oCheck**® Test Kit has been validated using the Uracil-N-Glycosylase from Thermo Scientific (see chapter 2). It is mandatory to use this enzyme in order to achieve the claimed performance.

- Add 1 µL of this dilution to each Papill**oCheck**® PCR reaction (see chapter 8.2.3, Table 4).

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 Papill**oCheck**®.

In general, for UNG treatment the PCR 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 Papill**oCheck**® PCR and correspond to the first two steps of the thermal cycler program (see Table 3). Within the second step (15 minutes at 95 °C) both inactivation of the Uracil-N-Glycosylase and activation of the HotStarTaq® Polymerase occur.

> **!** The incorporated UNG system of the Papill**oCheck**® 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.

8.2.3 PCR reaction setup

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

Generally, the Papill**oCheck**® PCR setup procedure can be divided into two parts: the preparation of the final mastermix by adding enzymes to the Papill**oCheck**® PCR MasterMix and the preparation of the final PCR reaction by adding extracted DNA as template.

> **!** The Papill**oCheck**® Test Kit has been validated using HotStarTaq® Polymerase from Qiagen (see chapter 2). It is mandatory to use this enzyme in order to achieve the claimed performance.

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

- Prepare the final mastermix (consisting of Papill**oCheck**® PCR MasterMix, HotStarTaq® Polymerase

and Uracil-N-Glycosylase) for the required quantity of PCR reactions as outlined in Table 4.

To analyse multiple samples, the final 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 mastermix volume for 13 amplification reactions if 12 samples are to be tested (see Table 4).

> ! It is recommended to include a negative control for every batch of final 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 mastermix thoroughly by either vortexing for 2 seconds and then spinning down or by pipetting up and down several times.
- Aliquot the final mastermix by pipetting 21 µL for each PCR reaction into a 0.2 mL, thin-walled PCR reaction tube.

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

- For the preparation of the reaction mix, add 5 µL of DNA extract to each final mastermix 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 PCR reaction is 26 µL.
- Place the reaction tubes in the thermal cycler and start the PCR reaction using the thermal cycler program described in chapter 8.2.1 (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.

Table 4: Setup of the PapilloCheck® PCR reaction

	1 reaction	13 reactions (1 chip)	26 reactions (2 chips)	39 reactions (3 chips)	52 reactions (4 chips)
PapilloCheck® PCR MasterMix	19.8 µL	257.4 µL	514.8 µL	772.2 µL	1029.6 µL
HotStarTaq® DNA Polymerase (5 U/µL)	0.2 µL	2.6 µL	5.2 µL	7.8 µL	10.4 µL
Uracil-N-Glycosylase (Dilution of 1:200, 0.005 U/µL)	1 µL	13µL	26 µL	39 µL	52 µL
Total volume before addition of sample DNA (final mastermix)	21 µL	273 µL	546 µL	819 µL	1092 µL
DNA extract from cervical sample (DNA template)	5 µL				
Total volume per reaction (final PCR reaction)	26 µL				

8.3 Hybridisation and washing

8.3.1 Preparation and setup

Begin with the necessary preparations for the hybridisation an washing steps at least 30 minutes prior to starting the hybridisation procedure to ensure that all needed components are equilibrated to room temperature.

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

Storage of the Papill**oCheck**® Test Kit at 4 to 8 °C may result in precipitation of SDS in the Hybridisation Buffer and Buffer B. Allow the solutions to equilibrate to room temperature and then vortex the tube or agitate 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 Papill**oCheck**® analysis is available from Greiner Bio-One (see chapter 2).

- Incubate the required amount of Papill**oCheck**® chips in the prepared Hybridisation Chamber at room temperature for at least 10 minutes.

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

- Prepare the washing solutions I, II and III according to the following instructions.

Preparation of washing solutions I, II and III:

- Prepare the washing solution mix for washing solutions I, II and III appropriate for the number of Papill**oCheck**® chips being analysed as shown in Table 5.
- Aliquot three equal volumes of the washing solution mix into three separate oCheck® WashBoxes and label them as washing solution I, II and III. 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

	Number of Papill**oCheck**® chips			
Components	**1**	**2**	**3**	**4**
Purified water	140 mL	280 mL	420 mL	560 mL
Papill**oCheck**® Buffer A	14 mL	28 mL	42 mL	56 mL
Papill**oCheck**® Buffer B	1.75 mL	3.5 mL	5.25 mL	7 mL
Total volume	155.75 mL	311.50 mL	467.25 mL	623 mL

! Never reuse the washing solutions as this could lead to an accumulation of washed-off PCR product that possibly interferes with PapilloCheck® results. Use fresh washing solutions for each assay.
The prepared washing solution mix can be stored up to one week at room temperature. Check if precipitation of SDS has occured. If so, warm up the washing solution mix until the precipitate is dissolved and equilibrate to room temperature again. Then prepare for the next hybridisation experiment.

8.3.2 Hybridisation

Hybridisation must be performed at ambient temperature between 20 and 25 °C. The principle working steps for hybridising the PCR products of the Papill**oCheck**® PCR reaction onto the Papill**oCheck**® chip are shown in Figure 5.

- 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 Hybridisation Buffer before use. Briefly spin down.
- Transfer 30 µL of the Papill**oCheck**® Hybridisation Buffer in a fresh reaction tube of an 8x PCR strip. If more convenient, also a new well of a PCR plate can be used.
- Add 5 µL of the PCR product to the Hybridisation Buffer and mix by either vortexing or by pipetting up and down several times.
- Briefly spin down.
- Transfer 25 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 (see Figure 5). 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.

! Avoid formation of bubbles!

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.

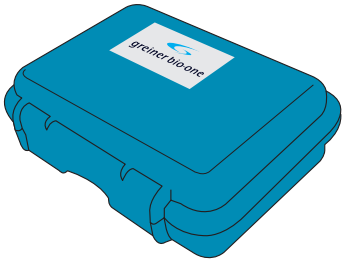
! Handle the chip carefully to avoid spilling of the hybridisation mix. Spilling can lead to cross-contamination of samples and to false positive results.

- Incubate the chip for exactly 15 minutes at ambient temperature between 20 and 25 °C within the prepared Hybridisation Chamber in a dark, humidity-saturated atmosphere. Be careful not to move the Hybridisation Chamber during the hybridisation.

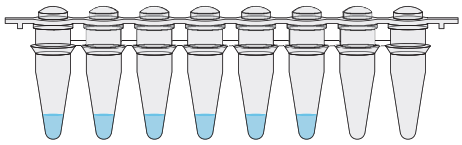
! Never change the incubation time or temperature of the hybridisation reaction, as this may cause a loss of fluorescence signal intensity or an increase in unspecific fluorescence.

Do not expose the hybridised chips to direct sunlight.

- Prepare a humidity-saturated atmosphere in the Hybridisation Chamber.
- Incubate the required amount of Papill**oCheck**® chips in the Hybridisation Chamber at room temperature (see chapter 8.3.1).



- Mix 30 µL of the Papill**oCheck**® Hybridisation Buffer in a 0.2 mL reaction tube of a PCR strip with 5 µL of the PCR product. Mix thoroughly.



- Transfer 25 µL of hybridisation mix into each well of the Papill**oCheck**® chip using a multichannel pipette.

- Close the Hybridisation Chamber and incubate the Papill**oCheck**® chip for exactly 15 minutes at ambient temperature between 20 and 25 °C.

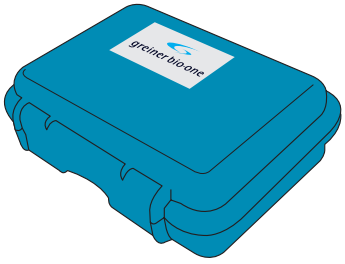


Figure 5: Working steps of the hybridisation procedure

8.3.3 Washing and drying

Special equipment supplied by Greiner Bio-One enables the parallel washing of up to four Papill**oCheck**® chips (see chapter 2). The additional equipment required for processing the Papill**oCheck**® chips is comprised of three oCheck® WashBoxes and a handle for the magnetic slideholder of the Hybridisation Chamber.

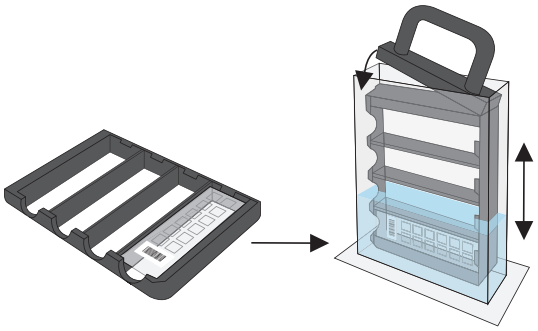
The different working steps are shown in Figure 6.

- 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 three washing steps.
- Wash the chip at room temperature in washing solution I by moving it quickly up and down for 10 seconds. The arrays must stay covered with washing solution at all times.
- Wash the chip for 60 seconds in washing solution II at 50 °C by vigorously moving the slideholder up and down.
- Wash the chip at room temperature in washing solution III by moving it quickly up and down for 10 seconds.
- 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 Papill**oCheck**® chip into a 50 mL tube and centrifuge at room temperature for 3 minutes at 500 x g.

The Papill**oCheck**® 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.

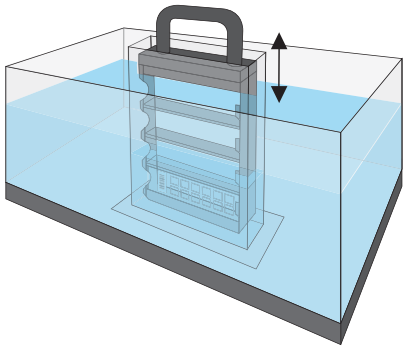
First washing step

- 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 Papill**oCheck**® chip(s) in washing solution I at room temperature for 10 seconds by moving the slideholder up and down



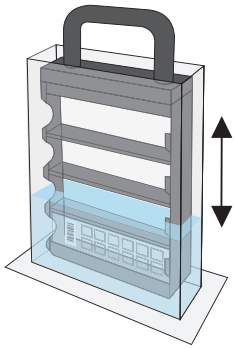
Second washing step

- Wash the Papill**oCheck**® chip(s) in washing solution II in a water bath 50 °C for 60 seconds by moving the slideholder up and down.



Third washing step

- Wash the Papill**oCheck**® chip(s) in washing solution III at room temperature for 10 seconds by moving the slideholder up and down



Drying

- Immediately remove any liquid from the surface of the Papill**oCheck**® chips by centrifugation

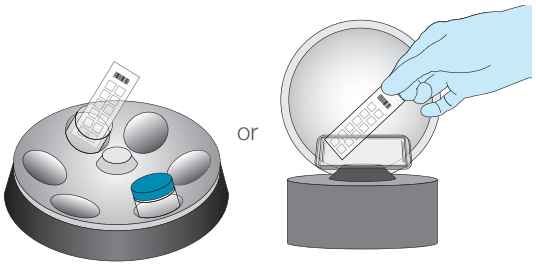


Figure 6: Working steps of the washing procedure
Different washing steps and drying procedure prior to the analysis of the Papill**oCheck**® chip with the CheckScanner™ and the CheckReport™Software.

8.4 Scanning and evaluation of the PapilloCheck® chip

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

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 Papill**oCheck**® kit. If the versions do not match, update the CheckReport™Software. The latest Software version can be downloaded from the Greiner Bio-One website:
www.gbo.com ➠ Know-How & Services ➠ Download-Center

9. TROUBLESHOOTING

If one of the following error messages occurs during chip scanning or if the Papill**oCheck**® analysis fails due to specific on-chip controls, proceed as described below. Please do not hesitate to contact your local Greiner Bio-One distributer if you have any questions or experience any difficulties while using Papill**oCheck**®.

For troubleshooting problems with other assay steps, e.g. DNA extraction, please refer to the respective Instructions For Use.

PROBLEM and cause	Comments and suggestions
ERROR MESSAGE „COULD NOT READ BARCODE“	
Damaged barcode	Check barcode for damage. Enter the barcode manually when the appropriate window appears.
Chip was not loaded correctly	Check chip orientation and scan the chip in correct orientation.
ERROR MESSAGE „MISSING SPOTS“, PRINTING CONTROL FAILED OR ORIENTATION CONTROL FAILED	
Dust on the chip	Repeat hybridisation of PCR product(s) on another chip.
Formation of air bubbles during transfer of liquid on the chip	Repeat hybridisation of PCR product(s) on another chip. Pipette carefully to avoid air bubble formation.
HYBRIDISATION CONTROL FAILED	
Incorrect temperature of washing solution II	The second washing step must be performed at 50 °C. Ensure that the washing solution II is heated to 50 °C.
Incorrect temperature of water bath	The second washing step must be performed at 50 °C. Check the temperature of the water bath. Ensure that the water bath is set to a temperature of 50 °C. If necessary, confirm temperature using a thermometer.
Wrong preparation of hybridisation mix	Repeat preparation of hybridisation mix with the correct volumes and hybridise PCR products on another chip.

PROBLEM and cause	Comments and suggestions
PCR CONTROL FAILED	
No addition of HotStarTaq® DNA Polymerase to the PapilloCheck® PCR MasterMix	Repeat PapilloCheck® analysis starting with the preparation of the PCR reaction.
Addition of a not proper functioning HotStarTaq® DNA Polymerase to the PapilloCheck® PCR MasterMix	Repeat PapilloCheck® analysis starting with the preparation of the PCR reaction.
Addition of undiluted Uracil-N-Glycosylase to the PapilloCheck® PCR MasterMix	Repeat PapilloCheck® analysis starting with the preparation of the PCR reaction.
Insufficient mixing of final mastermix	Repeat PapilloCheck® analysis starting with the PCR reaction. Take care to mix the reaction mix thoroughly.
PCR inhibitors are present in the sample	Repeat DNA extraction and PapilloCheck® analysis.
Hybridisation was performed without addition of PCR product	Repeat hybridisation.
Insufficient mixing of final hybridisation mix	Repeat hybridisation.
Problems with the thermal cycler	Only use validated thermal cyclers in combination with PapilloCheck®.
SAMPLE CONTROL FAILED	
No addition of sample DNA to the final mastermix	Repeat PapilloCheck® analysis starting with the PCR reaction.
DNA preparation failed	Repeat DNA extraction.
Not enough sample material	Failed sample collection. Specimen is very clear. Concentrate specimen according to the description in chapter 8.1 and repeat DNA extraction or repeat sample collection.
PCR AND/OR SAMPLE CONTROLS HAVE NOT FAILED BUT DISPLAY A SNR VALUE OF 0	This result is rated as valid if the CheckReport™Software detects at least one HPV type in the sample with a signal above a defined threshold. Sample and/or PCR control may then show low or even absent fluorescence signals due to competition during PCR.

10. TECHNICAL SUPPORT

If you have any questions, experiences or difficulties concerning Greiner Bio-One 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.

11. PERFORMANCE CHARACTERISTICS OF PAPILLOCHECK®

11.1 Analytical performance of PapilloCheck®

11.1.1 Analytical sensitivity

For assessing the analytical sensitivity, the limit of detection (LoD95 = smallest number of copies of target DNA which can be detected in 95% of cases) was established using reference plasmids for each detectable HPV type containing the E1 region targeted by Papill**oCheck**®. For the analysis the HPV genotype specific plasmids were spiked in transport medium including cells from a human cell line (HEK cells) as background. DNA was extracted from each sample and subsequently analysed with the Papill**oCheck**® Kit. The LoD95 was calculated for each HPV genotype individually using 2 independent dilution series and Probit analysis. The defined LoD95 was verified with a third dilution series for each HPV genotype. The limits of detection for the different HPV genotypes are summarised in Table 6.

Table 6: Analytical sensitivity of PapilloCheck®

HPV Genotype*	Limit of Detection (LoD95) for PapilloCheck® [copies/PCR]**	Limit of Detection (LoD95) for PapilloCheck® [copies/extraction]***
HPV 6	30	600
HPV 11	20	400
HPV 16	10	200
HPV 18	320	6400
HPV 31	50	1000
HPV 33	90	1800
HPV 35	190	3800
HPV 39	50	1000
HPV 40	20	400
HPV 42	50	1000
HPV 43	30	600
HPV 44	20	400
HPV 45	90	1800
HPV 51	20	400
HPV 52	70	1400
HPV 53	60	1200
HPV 55	20	400
HPV 56	50	1000
HPV 58	160	3200
HPV 59	290	5800
HPV 66	40	800
HPV 68	30	600
HPV 70	30	600
HPV 73	2250	45000
HPV 82	40	800

* HPV genotype specific plasmid with 10^4 human HEK cells as background in PreservCyt™ (Hologic).
** LoD95 per PCR reaction calculated from LoD95 per extraction (see ***)
*** LoD95 based on probit analysis including recommended oCheck® DNA Extraction system from Greiner Bio-One

11.1.2 Analytical specificity – HPV types

Papill**oCheck**® has been tested regarding analytical specificity using HPV genotype specific reference plasmids. The following HPV genotypes were tested with 10^5 copies/PCR reaction:

HPV 6, HPV 11, HPV 16, HPV 18, HPV 26, HPV 30, HPV 31, HPV 33, HPV 34, HPV 35, HPV 39, HPV 40, HPV 42, HPV 43, HPV 44, HPV 45, HPV 51, HPV 52, HPV 53, HPV 54, HPV 55, HPV 56, HPV 58, HPV 59, HPV 61, HPV 66, HPV 67, HPV 68, HPV 69, HPV 70, HPV 71, HPV 73, HPV 81, HPV 82, HPV 84, HPV 85.

The following cross-hybridisations were detected:

HPV 55 gives a signal on the HPV 44 probe. As a result, the CheckReport™Software displays a combined HPV 44/HPV 55 result. HPV 13 may cross-react with the HPV 11 probe but does not result in a false positive signal as HPV 13 is not present in cervical specimens.

11.1.3 Analytical specificity – Non-HPV organisms

Papill**oCheck**® has been tested regarding analytical specificity using the following non-HPV organism (1ng genomic DNA/PCR reaction):

Bacteroides ureolyticus, Bacteroides uniformis, Bifidobacterium breve, Bifidobacterium adolescentis, Chlamydia trachomatis, Clostridium difficile, Clostridium perfringens, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter sakazakii, Enterobacter faecium, Enterobacter durans, Enterobacter faecalis, Escherichia coli, Fusobacterium nucleatum, Klebsiella oxytoca, Klebsiella pneumoniae, Neisseria gonorrhoeae, Peptostreptococcus anaerobius, Peptostreptococcus micros, Proteus hauseri, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Trichomonas vaginalis, Candida albicans

No unspecific signals were detected with Papill**oCheck**®**.**

11.2 Repeatability (Intra-Reproducibility)

The repeatability measures the performance variation between different runs in one laboratory. For assessing test repeatability DNA from 4 samples, each containing a different HPV template (HPV 16, HPV 18, HPV 31 or HPV 45) in human cell background was extracted using the manual oCheck® DNA Extraction Kit and analysed with the Papill**oCheck**® Kit. Each sample was analysed in 6 independent replicates and in 3 independent runs. Hence, 18 replicates from each sample and altogether 72 samples were tested per repeatability evaluation. All samples were tested by the same user, with the same equipment and kit lots.
Additionally, the described repeatability evaluation was performed at 3 test sites, so in total 216 analyses were carried out. An agreement of 100% was obtained for each of the 3 repeatability testings (see Table 7).

Table 7: Results of the repeatability testings with Papill**oCheck**®

Test Site	Agreement
Test site 1	100 %
Test site 2	100 %
Test site 3	100 %

11.3 Reproducibility

The reproducibility measures the performance variation between different runs in different laboratories. For assessing test reproducability, again DNA from 4 samples, each containing a different HPV template (HPV 16, HPV 18, HPV 31 or HPV 45) in human cell background was extracted using the manual oCheck® DNA Extraction Kit and analysed with the Papill**oCheck**® Kit. The procedure was carried out in parallel at 3 different test sites. Each sample was analysed in 6 independent replicates and 3 independent runs at each test site. Hence, 18 replicates from each sample and altogether 72 samples were tested per test site. Each test site used different equipment (PCR cycler, CheckScanner™) and different kit lots. In total 216 samples were tested. An agreement of 100% was obtained for the results of the different test sites.

11.4 Robustness

In order to evaluate the robustness of the Papill**oCheck**® test system variations of the following parameters were considered:

- Hybridisation temperature
- Hybridisation time
- Washing temperature

All tests were performed in 3 replicates with high template concentrations (10^5 copies/PCR reaction of HPV reference plasmid) and with a template concentration near the detection limit (concentration depending on HPV genotype). The tests were performed for each HPV genotype detectable with Papill**oCheck**®. The ranges of parameter values determined in which a robust HPV detection is possible are summarised in Table 8.

Table 8: Papill**o**Check® Robustness

Parameter	Range
Hybridisation temperature	18-25 °C
Hybridisation time	13-17 minutes
Washing temperature	48-52 °C

11.5 Clinical Performance of PapilloCheck®

To determine the clinical performance of the Papill**oCheck**® and Papill**oCheck**® high-risk assay in terms of clinical sensitivity and specificity, a comparative study was performed using Papill**oCheck**® and the GP5+/6+-PCR EIA assay[12]. For this study, samples of 1,437 representative women with normal cytology (control group) over 40 years of age (median age, 49 years; age range, 40 to 60 years) and 192 representative women (median age, 34 years; age range, 30 to 60 years) with histologically confirmed CIN3+ lesions (case group) were analysed. All samples used in this study were originally collected during the baseline round from women in the intervention group of the population-based randomised-controlled implementation trial POBASCAM[13].

After restricting the Papill**oCheck**® analysis to the 14 hrHPV types targeted by GP5+/6+-PCR-EIA, and the new Papill**oCheck**® high-risk, Papill**oCheck**® had a clinical sensitivity for ≥ CIN3 of 95.8 % (184/192; 95 % CI 92.8-98.8) and a clinical specificity for ≥ CIN2 of 96.7 % (95 % CI 95.7-97.7). By comparison, these figures were 96.4 % (185/192; 95 % CI: 93.9-98.9) and 97.7 % (95 % CI: 96.9-98.5), respectively, for GP5+6+- PCR-EIA (see Table 9 and 10).

Table 9: Comparison of Papill**oCheck**® (14 hr HPV types as targeted by Papill**oCheck**® high-risk) and GP5+/6+ PCR-EIA results stratified for controls and cases.

	Papill**oCheck**® (14 hrHPV types)	GP5+/6+-PCR/EIA		Total
		-	+	-
Controls	- + total	1,386 (96.5 %) 18 (1.3 %) 1,404 (97.7 %)	4 (0.3 %) 29 (2.0 %) 36 (2.3 %)	1,390 (96.7 %) 47 (3.3 %) 1,437
Cases	- + total	4 (2.1 %) 3 (1.6 %) 7 (3.6 %)	4 (2.1 %) 181 (94.3 %) 185 (96.4 %)	8 (4.2 %) 184 (95.8 %) 192

Table 10: Clinical sensitivity and specificity of Papill**oCheck**® and the GP5+/6+-PCR-EIA (results for one or more of the 14 hr HPV types HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68).

14 hrHPV	Papill**oCheck**®	GP5+/6+
Clinical Sensitivity for ≥ CIN3	95.8 %	96.4 %
Clinical Specificity for ≥ CIN2	96.7 %	97.7 %

[12] Comparison of the clinical performance of PapilloCheck® human papillomavirus detection with that of the GP5+/6+-PCR-enzyme immunoassay in population-based cervical screening. Hesselink AT, Heideman DA, Berkhof J, Topal F, PolRP, Meijer CJ, Snijders PJ. J Clin Microbiol. 2010 Mar;48(3):797-801. Epub 2009 Dec 30.

[13] Bulkmans, N. W., L. Rozendaal, P. J. Snijders, F. J. Voorhorst, A. J. Boeke, G. R. Zandwijken, F. J. van Kemenade, R.H. Verheijen, K. Groningen, M. E. Boon, H. J. Keuning, M. van Ballegooijen, A. J. van den Brule, and C. J. Meijer. 2004. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. Int. J. Cancer 110:94–101.

To assess the reproducibility of the Papill**oCheck**® assay with clinical samples, a study was carried out in 2012 in the Netherlands and Germany. In the study cervical specimens obtained from a cohort of 10,000 women who participated in regular cervical cytology screening in the Utrecht province of the Netherlands were used. Using a defined set of 550 pretested samples Papill**oCheck**® showed an intra-laboratory reproducibility and inter-laboratory agreement of 97,6% and 94%, respectively. The data sets of the reproducibility evaluation are summarised in Table 11 and 12.

Table 11: Intra-Laboratory Reproducibility

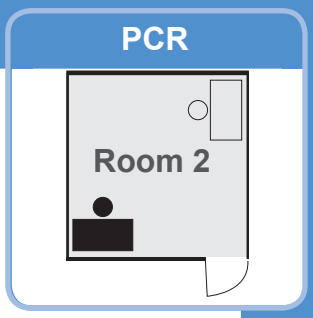
	Papill**oCheck**® time 1 pos	Papill**oCheck**® time 1 neg
Papill**oCheck**® time 2 pos	147	6
Papill**oCheck**® time 2 neg	7	390

Results:
Reproducibility [%]: 97.3
Lower confidence bound [%] = 96.3
Kappa = 0.941

Table 12: Inter-Laboratory Agreement

	Papill**oCheck**® time 1 pos	Papill**oCheck**® time 1 neg
Papill**oCheck**® time 2 pos	123	2
Papill**oCheck**® time 2 neg	31	394

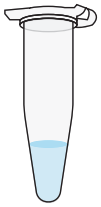
Results:
Reproducibility [%]: 94
Lower confidence bound [%] = 92.1
Kappa = 0.842



12. PAPILLOCHECK® SHORT PROTOCOL

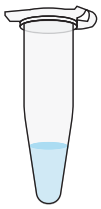
12.1 Room 2: PCR-setup of reaction mix

- Dilute the Uracil-N-Glycosylase 1:200 in PCR-grade water
- Mix the UNG dilution carefully



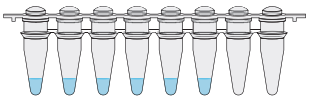
- Prepare the final mastermix for the required quantity of PCR reactions

	1 reaction	13 reactions (1 chip)	26 reactions (2 chips)	39 reactions (3 chips)	52 reactions (4 chips)
PapilloCheck® PCR MasterMix	19.8 µL	257.4 µL	514.8 µL	772.2 µL	1029.6 µL
HotStar Taq® Polymerase (5 U/µL)	0.2 µL	2.6 µL	5.2 µL	7.8 µL	10.4 µL
Uracil-N-Glyco-sylase (Dilution of 1:200, 0.005 U/µL)	1 µL	13µL	26 µL	39 µL	52 µL
Total volume before addition of sample DNA	21 µL	273 µL	546 µL	819 µL	1092 µL



- Mix the final mastermix carefully

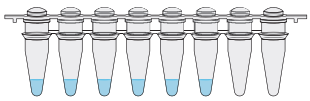
- Aliquot the final mastermix: add 21 µL of the reaction mix for each PCR reaction into a 0.2 mL PCR reaction tube of a PCR strip.



SHORT PROTOCOL

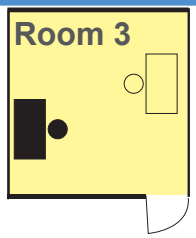
12.2 Room 2: PCR-DNA template addition / PCR reaction

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



- 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 25 s 45 s	95 °C 55 °C 72 °C	40
30 s 45 s	95 °C 72 °C	15
Hold	10 °C	



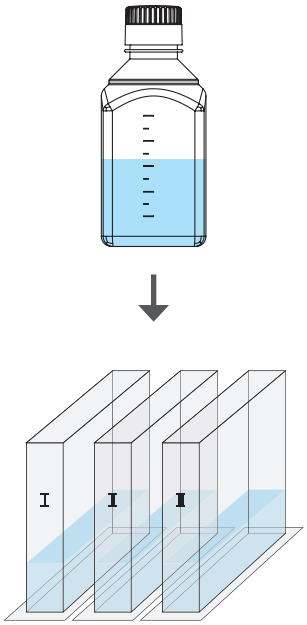
12.3 Room 3: Hybridisation - Preparation / Hybridisation reaction

! Begin preparations at least 30 minutes prior to hybridisation.

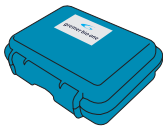
- Dissolve potential precipitates in the hybridisation and washing buffers and mix well
- Prepare the washing solution mix for the amount of Papill**oCheck**® chips to be analysed.

Components	Number of PapilloCheck® chips			
	1	2	3	4
Distilled/deionised water	140 mL	280 mL	420 mL	560 mL
PapilloCheck® Buffer A	14 mL	28 mL	42 mL	56 mL
PapilloCheck® Buffer B	1.75 mL	3.5 mL	5.25 mL	7 mL
Total volume	155.75 mL	311.50 mL	467.25 mL	623 mL

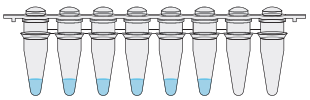
- Aliquot the washing solution mix into three oCheck® WashBoxes
- Preheat washing solution II in a water bath at 50 °C



- Incubate the amount of Papill**oCheck**® chips to be analysed in the prepared Hybridisation Chamber at room temperature



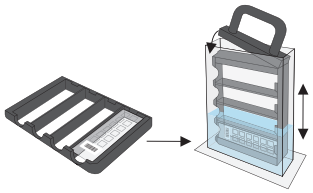
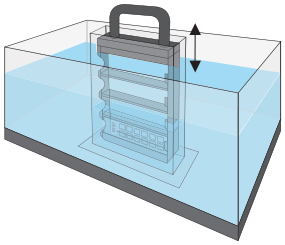
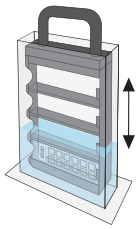
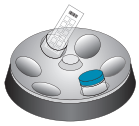
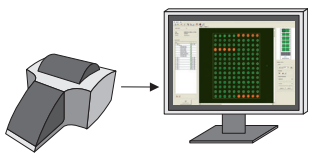
- Mix PCR products and briefly spin down
- Mix Hybridisation Buffer and briefly spin down
- Mix 30 µL Papill**oCheck**® Hybridisation Buffer with 5 µl PCR product
- Mix thoroughly and briefly spin down



- Transfer 25 µL of the hybridisation mix into each well of the Papill**oCheck**® chip using a multichannel pipette
- Avoid air bubble formation

- Incubate the Papill**oCheck**® chip for exactly 15 minutes at ambient temperature 20 to 25 °C

12.4 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 Papill**oCheck**® chip(s) in washing solution I at room temperature for 10 seconds	
➠ Wash the Papill**oCheck**® chip(s) in preheated washing solution II in a water bath at 50 °C for 60 seconds	
➠ Wash the Papill**oCheck**® chip(s) in washing solution III at room temperature for 10 seconds	
➠ Remove any liquid from the Papill**oCheck**® chip surface by centrifugation	 3 minutes 500 g or  1 minute max. speed
➠ Scan the Papill**oCheck**® chip(s) with the CheckScanner™ ➠ Perform scanning and analysis as described in the User Guide of the CheckReport™Software ➠ Create reports	

SHORT PROTOCOL