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1 Introduction

- ParoCheck™ is the first DNA chip for the detection of periodontitis associated pathogens. ParoCheck™ was developed in a co-operation between Lambda and Greiner Bio-One and allows the rapid and exact determination of a total of 10 or 20 different periodontal pathogens (see below). ParoCheck™ thus enables an as yet unique monitoring of pathogens in all areas of periodontal disease, such as:
  - juvenile periodontitis
  - chronic adult periodontitis
  - aggressive periodontitis
  - refractory, marginal periodontitis

### With ParoCheck® Kit 20 Detectable Periodontal Pathogens

<table>
<thead>
<tr>
<th>Pathogen (Actinobacillus actinomycetemcomitans)</th>
<th>Pathogen (Campylobacter rectus/showae)</th>
<th>Pathogen (Porphyromonas gingivalis)</th>
<th>Pathogen (Treponema denticola)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces odontolyticus</td>
<td>Capnoclopatha gingivalis/spitgena/ochraceae</td>
<td>Prevotella intermedia</td>
<td>Veillonella parvula</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>Eikenella corrodes</td>
<td>Prevotella nigrescens</td>
<td></td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>Eubacterium nodatum</td>
<td>Streptococcus constellatus group*</td>
<td></td>
</tr>
<tr>
<td>(synonym: Bacteroides forsythus; Tannerella forsythensis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter concinis</td>
<td>Fusobacterium nucleatum</td>
<td>Streptococcus gordonii group*</td>
<td></td>
</tr>
<tr>
<td>Campylobacter gracilis</td>
<td>Peptostreptococcus micro</td>
<td>Streptococcus mitis group*</td>
<td></td>
</tr>
</tbody>
</table>

### With ParoCheck® Kit 10 Detectable Periodontal Pathogens

<table>
<thead>
<tr>
<th>Pathogen (Actinobacillus actinomycetemcomitans)</th>
<th>Pathogen (Campylobacter rectus/showae)</th>
<th>Pathogen (Porphyromonas gingivalis)</th>
<th>Pathogen (Treponema denticola)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces odontolyticus</td>
<td>Capnoclopatha gingivalis/spitgena/ochraceae</td>
<td>Prevotella intermedia</td>
<td>Veillonella parvula</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>Eikenella corrodes</td>
<td>Prevotella nigrescens</td>
<td></td>
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<tr>
<td>Tannerella forsythia</td>
<td>Eubacterium nodatum</td>
<td>Streptococcus gordonii group*</td>
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</tr>
<tr>
<td>(synonym: Bacteroides forsythus; Tannerella forsythensis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>Peptostreptococcus micro</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three probes present on the ParoCheck® Kit 20 are specific for groups of Streptococcus species. The three groups are:

<table>
<thead>
<tr>
<th>Streptococcus group</th>
<th>Species detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. constellatus group</td>
<td>S. constellatus, S. intermedius, S. anginosus</td>
</tr>
<tr>
<td>S. mitis group</td>
<td>S. mitis, S. oralis, S. sanguinis, S. parasanguinis</td>
</tr>
<tr>
<td>S. gordonis group (mutans group)</td>
<td>S. gordonis, S. salivarius, S. cricetus, S. mutans, S. s. ubens</td>
</tr>
</tbody>
</table>

1.1 Assay Principle

The principle of the assay outlined in figure 1 is based on the detection of the pathogen-specific 16S rRNA gene. After taking a patient specimen, the bacterial DNA is extracted (specimen collection and DNA extraction kits are not provided). Subsequently a DNA fragment of about 300 nucleotides, coding for the 16S rRNA genes of microorganisms existing in the specimen, is amplified in the presence of only one highly conserved pair of primers with the aid of the polymerase chain reaction. The polymerase chain reaction was designed in such a way that single-stranded DNA fragments are formed, which are fluorescence-labeled with Cy5. The labeled amplified fragments are then hybridized to pathogen-specific DNA probes derived from the area of the 16S rRNA gene and fixed to the periodontitis DNA chip. After hybridization, analysis can be performed with any microarray scanner which is able to handle the dimensions of a microscopic slide (25 mm x 75 mm). The following wavelengths are required for detection: ~532 nm (Cy3) and ~635 nm (Cy5).

Using the CheckScanner™ and the CheckReport™ software, described in section 4., the evaluation and creation of the ‘ParoCheck® report’ are easy and fast. Automated interpretation of the on-chip controls and perfect data administration fulfill all needs required for a diagnostic set-up.

Optional, images generated by an Axon 4000A, 4000B or 4100A personal scanner can be imported and analyzed using the CheckReport™ software.

---

1 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.
**1.2 Assay Procedure**

I. **PCR**

After extraction of bacterial DNA, a fragment of about 300 nucleotides, encoding part of the 16S rRNA gene of any microorganism existing in the specimen, is amplified with the aid of the polymerase chain reaction (PCR) in the presence of one highly conserved primer pair. The PCR design leads to single-stranded DNA fragments which are fluorescence-labeled with Cy5 molecules.

II. **Hybridization**

The labeled probes are then hybridized to complementary sequences fixed on the chip. Each periodontal pathogen is detected by five measuring points on the chip. During subsequent washing steps improper bound probes and probes in excess are washed out.

III. **Scan /Analysis**

The bound and labeled probes are detected by stimulation with monochromatic light. The analysis of bound probes is then carried out using the CheckReport™ analysis-software.

**Fig. 1: Principle of the ParoCheck® assay**

I. After extraction of bacterial DNA, a fragment of about 300 nucleotides, encoding part of the 16S rRNA gene of any microorganism existing in the specimen, is amplified with the aid of the polymerase chain reaction (PCR) in the presence of one highly conserved primer pair. The PCR design leads to single-stranded DNA fragments which are fluorescence-labeled with Cy5 molecules.

II. The labeled probes are then hybridized to complementary sequences fixed on the chip. Each periodontal pathogen is detected by five measuring points on the chip. During subsequent washing steps improper bound probes and probes in excess are washed out.

III. The bound and labeled probes are detected by stimulation with monochromatic light. The analysis of bound probes is then carried out using the CheckReport™ analysis-software.

**1.3 Set-up of the ParoCheck® 20 DNA chip**

The chip has 12 wells, A1 – B6, with a printable area of 6x6 mm each. Each well contains one ParoCheck® microarray consisting of 120 DNA measuring points distributed over an area of about 8 mm².

**Optional for Art.No. 460 020:** Only the wells A1 and B1 contain a ParoCheck® microarray.

The DNA measuring points are about 150 µm in diameter and have a distance of 275 µm from each other. Three internal control systems enable the monitoring of the hybridization and PCR reaction as well as the determination of presence and homogeneity of each individual DNA spot.

Due to the use of fluorophore-labeled PCR primers (Cy5) and fluorophore-labeled targets for the internal control systems (Cy3), the analysis of the DNA chips is performed by microarray scanners using wavelengths of ~ 532 nm (Cy3) and ~ 635 nm (Cy5).

**1.3.1 Signals at wavelength ~ 532 nm (green fluorescence)**

1.3.1.1 Printing control

Each spot of the microarray gives a signal at wavelength ~ 532 nm. A Cy3-labeled probe in the hybridization buffer hybridizes with each measuring point, thus enabling a monitoring of presence and homogeneity of all DNA measuring points.

1.3.1.2 Hybridization control

Additionally, the Cy3-labeled probe in the hybridization buffer will react with an adequate complementary DNA sample, which is spotted in 5 measuring points. This allows to control the performance of the hybridisation reaction.

1.3.1.3 Orientation control

10 measuring points give a signal at wavelength ~ 532 nm independently of hybridisation. They allow to control the orientation of the analysis grid on the array.

**1.3.2 Signals at wavelength ~ 635 nm (red fluorescence)**

1.3.2.1 Internal PCR control

The validation of PCR is performed using 5 DNA measuring points. The binding of the Cy5-labeled PCR-product of an internal PCR control-template to five DNA measuring points ensures the evaluation of the PCR quality. In case of a lot of bacterial DNA present in a patient sample, signals on the PCR control points may decrease or even disappear due to primer competition during PCR. If so, one of the bacteria has to exceed a certain threshold so that a report will be generated. If the internal control and the bacteria fail to exceed the threshold, the results will not be released.

1.3.2.2 Detection of bacteria

Bacterial DNA is detected by the hybridization of the Cy5-labeled PCR amplification products to specific DNA probes spotted in replicas of 5 spots.
### 1.3.3 Order of the measurement points for the bacteria

Each bacterium is detected by five adjacent measurement points.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus actinomycetemcomitans</td>
<td>Actinomyces odontolyticus</td>
</tr>
<tr>
<td>Actinomyces viscosus</td>
<td>Campylobacter gracilis</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>Eubacterium nodatum</td>
</tr>
<tr>
<td>Campylobacter rectus/showae</td>
<td>Treponema denticola</td>
</tr>
<tr>
<td>Veillonella parvula</td>
<td>Streptococcus constellatus group</td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>Prevotella intermedia</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>Streptococcus mitis group</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>Prevotella nigrescens</td>
</tr>
<tr>
<td>Porphyromonas endodontalis</td>
<td>Streptococcus gordonii group</td>
</tr>
</tbody>
</table>

---

**Fig. 2: Set up of the DNA chip**

ParoCheck™ 20 DNA chip, read at the wavelengths 532 nm (green) and 635 nm (red). Colors of both images are false colors and correspond to the signal intensities.

- **Green channel (532 nm)** = Cy3-labeled targets
- **Red channel (635 nm)** = Cy5-labeled targets

**On-chip control systems allow the exact quality determination:**

1. **Red:** Orientation controls (Cy3-labeled probes; 10 measuring points)
2. **Yellow:**
   a) Dotted area: Printing and homogeneity control of all DNA measuring points (Cy3-labeled target; 105 measuring points)
   b) Full line area: Hybridization controls (Cy3-labeled targets; 5 measuring points)
3. **Turquoise:** PCR controls (Cy5-labeled PCR products; 5 measuring points)
4. **Violet:** Positive samples (Cy5-labeled PCR products, 5 measuring points per each pathogen)
1.3.4 Automated analysis of on-chip controls

The ParoCheck® on-chip control system is analyzed with assistance of the CheckReport software. It interprets the control signals and restrains or releases the generation of an analysis-report. Details are described in section 4.

2 Components of the ParoCheck® Kits

The ParoCheck® Kit 20 is available in the following 2 packaging sizes:

(Art.No. 460 020) 5 HTA12 slides, each containing 2 ParoCheck® arrays, for the analysis of 10 samples:

- 1x plastic container with 5 ParoCheck® 20 chips, each chip containing 2 ParoCheck® microarrays. The 2 ParoCheck® microarrays are placed in the wells A1 and B1.
- 1x MasterMix, 270 µl (ready to use)
  Contains all components required for performing the PCR (Cy5 fluorescence-labeled specific primers, control DNA, buffers and nucleotides), except Taq-Polymerase.
- 1x Hybridization buffer, 420 µl (ready to use)
  Contains all components required for performing the hybridization (stringent buffer, probes for spot and hybridization control, labeled with Cy3).
- 2x Buffer A, 35 ml each (concentrate).
- 1x Buffer B, 15 ml (concentrate).

(Art.No. 460 120) 5 HTA12 slides, each containing 12 ParoCheck® arrays, for the analysis of 60 samples:

- 1x plastic container with 5 ParoCheck® 20 chips, each chip containing 12 ParoCheck® microarrays. One ParoCheck® array is placed in each of the 12 wells.
- 5x MasterMix, 270 µl each (ready to use)
  Contains all components required for performing the PCR (Cy5 fluorescence-labeled specific primers, control DNA, buffers and nucleotides), except Taq-Polymerase.
- 5x Hybridization buffer, 420 µl each (ready to use)
  Contains all components required for performing the hybridization (stringent buffer, probes for spot and hybridization control, labeled with Cy3).
- 2x Buffer A, 35 ml each (concentrate).
- 1x Buffer B, 15 ml (concentrate).

2.1 Components Required but not Provided

- Set for the specimen collection from the patients
  Components are available from Greiner Bio-One on demand: Art.No. 460 070
- Components for the extraction of bacterial DNA
  Recommendation: Roche Diagnostics, High Pure PCR Template Preparation Kit (Ordering number: 1 796 82)
  Sigma-Aldrich GenElute Bacterial Genomic DNA Kit (Ordering number: G1N-10/75/350)
- DNAse-free water
- Compressed air or nitrogen (minimum purity 4.6)
- Taq-Polymerase
  Recommendation: Sigma-Aldrich Taq-Polymerase (Ordering number: D 1806)
  Roche Taq DNA Polymerase (SU/µl) Ordering #: 1146165

2.2 Equipment Required

- Microcentrifuge for 1.5 ml reaction tubes
- PCR instrument
- Incubator or water bath (60°C)
- Hybridization chamber
- Microarray scanner
  Mandatory: Check Scanner, Greiner Bio-One
  or
  Axon 4000A, 4000B, Personal 4100A
- Micropipettes (variable from 1 - 1000 µl)
- 8-channel multipipette (variable from 5 µl – 100 µl)
- Sterile pipette tips:
  Recommendation: Greiner Bio-One tips
  1) 0.5 - 10 µl tips (transparent, Cat.-No.: 765280)
  2) 10 – 100 µl tips (yellow, Cat.-No.: 685280)
  3) 100 – 1000 µl tips (blue, Cat.-No.: 686280)
  4) 0.5 – 10 µl filter tips (Cat.-No.: 772288)
  5) 10 – 100 µl filter tips (Cat.-No.: 752288)
  6) 100 – 1000 µl filter tips (Cat.-No.: 750288)
- Reaction tubes
  Recommendation: Greiner Bio-One reaction tubes
  1) reaction tube 1.5 ml (Cat.-No.: 616201)

  Warning: Only the recommended polymerases have been tested during validation of the ParoCheck® Kit. Using other polymerases may lead to differing results.
2.3 Storage conditions

Although the shipment of the ParoCheck® kit is carried out at room temperature, all components must be stored at 4 to 8 °C and protected from light.

Important Note: Liquid components should be mixed well before use! Precipitation of SDS can occur in Hybridization Buffer and Buffer B. The precipitates will resuspend if buffers are mixed well at room temperature.

2.4 General Information for Handling DNA 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. Avoid direct contact with the hybridization zone on the chip surface.

Only the labeled side of the chip is intended for hybridization.

Important Note: Do not use any marker pens for the identification of DNA chips as they lead to unspecific fluorescence on the chip. Store chips protected from light.

2.5 Safety instructions

Buffer B contains > 10% sodium dodecyl sulfate (SDS) and is to be classified according to EEC directive 67/548 and 88/379 as hazardous.

Classification (SDS): Xn, harmful

1. Hazard identification
   Warnings: 20/22 Harmful by inhalation and if swallowed
   38 Irritating to skin
   Notes: Substance hazardous to water and ground water

2. First aid measures
   Eye contact: Wash eyes with water for at least 15 minutes.
   Skin contact: Wash off with water.
   Ingestion: Seek medical advice.
   Inhalation: Remove victim to fresh air, if malaise develops go to a physician.

3. Accidental release measures
   After spillage: Sweep up spilled substance carefully.
   Absorbent material: Not necessary.
   Damage limitation: Special measures to limit damage are not necessary.

4. Exposure controls/personal protection
   Respiratory protection: Filter against harmful dust in case of contact with the substance
   Eye protection: protective glasses
   Hand protection: protective gloves
   Hygiene: Wash hands before breaks and at the end of the work.

5. Toxicological information
   LD50 oral: 1286 mg/kg (rat)

3 Analysis

3.1 Working Steps and Expenditure of Time Involved

Figure 3 shows the different working steps as well as the time spent. The components for specimen collection from the patient and for the DNA extraction are not included in the ParoCheck® kit.

![Working steps by performing the ParoCheck® kits](image)

### Specimen Collection

(Components are not provided with the kit)

Specimen are collected by the dentist. Following aspects should be taken into account:

1. Supragingival plaques must be removed.
2. The specimen must be taken before starting any treatment.
3. 1-3 tips should be submerged deeply into the periodontal pocket and should remain there for about 10 seconds.
4. All tips used for the patient are transferred to one or more transport containers.
5. The containers should be labeled clearly.
3.3 Specimen Transport

All transport containers used are enveloped as recommended for biological agents and sent to the analyzing laboratory. Under these conditions, samples are stable for several days but should be processed within 4 working days. Samples should be kept at 4 °C, if stored for more than 4 days.

3.4 Preparation of Samples

(Components are not provided with the kit)

For the extraction of bacterial DNA, we recommend the kits mentioned under item 2.1. DNA should be as pure as possible and free from PCR inhibitors. It is recommended to measure the DNA by absorbance at 260 nm using a spectrophotometer and to adjust the DNA concentration within a range of 2 - 10 ng/µl. 1 µl extracted DNA is used for the amplification of the bacterial DNA.

3.5 Polymerase Chain Reaction (PCR)

(Taq-Polymerase is not provided with the kit)

Since with the aid of the PCR very low amounts of genomic material can be detected, the following aspects are of absolute importance:

- Use only sterile and single-use materials.
- Use filter tips to minimize the risk of aerosol contaminations.
- Preparation of samples and PCR should be performed in separated working areas.
- Wear protective gloves and change them frequently.

With the exception of the heat-stable polymerase, the MasterMix contains all components required for performing the PCR (buffers, MgCl₂, dNTPs, DNAse-free water and fluorophore-labeled primers). For achieving optimal results, the use of Taq-polymerase is recommended. The PCR is performed in a total volume of 20 µl using 0.2 ml thin walled PCR reaction tubes. For each reaction the components are mixed as outlined in table 1. If multiple samples should be analyzed, the reaction mix (consisting of MasterMix and Taq polymerase) can be prepared in a batch (i.e. in the quantity required for all analyses). It is recommended to perform a negative control for each PCR run by adding ultrapure water in place of bacterial DNA to one extra reaction. The negative control is then hybridized into any of the wells. There should be no signals at 635 nm except the internal PCR control.

Table 1:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacterial DNA (2-10 ng/µl)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>PCR MasterMix</td>
<td>18.8 µl</td>
</tr>
<tr>
<td>Taq-Polymerase (5 U/µl)</td>
<td>0.2 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

This amplification procedure is optimized for the use of the GeneAmp 9700 (Perkin Elmer). If possible, please set the reaction volume at the instrument used to 20 µl and amplify the bacterial DNA under the conditions listed in table 2.

Table 2:

<table>
<thead>
<tr>
<th>Time</th>
<th>Temp. °C</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>20 s</td>
<td>95</td>
<td>45</td>
</tr>
<tr>
<td>20 s</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>30 s</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Set the heating ramp to 1°C/s and the temperature of the lid to about 105 °C. After the PCR has been completed, the amplification products may be hybridized directly or stored for some days in the dark at 4 °C.

3.6 Hybridization

Important Note: Hybridization has to be performed at 60 °C as indicated. Variations in temperature exceeding ±3 °C may cause a loss in signal intensity or an increase in background fluorescence. Verify the hybridization temperature regularly! Since the hybridization volume (25 µl) is very small and a drying up of the hybridization solution on the chip has to be strictly avoided, it is necessary to perform the hybridization in a steam-saturated atmosphere. Handle the chip carefully to avoid the hybridization mix to flow over.

Preparation of washing buffers I, II, III

Important Note: Washing buffers have to be changed for each chip to avoid carry-over of signals! The kit contains a surplus of buffers A and B to use fresh buffer for each chip.

Mix 120 ml double-distilled water with 12 ml buffer A and 1.5 ml buffer B. Fill three approximately equal parts of this mix into three 50 ml reaction tubes and mark them as washing buffer I, II, and III. Preheat washing buffers I and II to 60 °C before use.
Perform the hybridization steps outlined in figure 4 as follows:

**Important Note:** For hybridizing up to 12 PCR-products on one chip it is strongly recommended to use an 8-channel multipipette and to handle always 6 samples at once. This makes handling faster and reduces the risk of drying.

1. Incubate the chip for at least 5 minutes at 60 °C in a steam-saturated atmosphere.

2. Mix 30µl of the hybridization buffer in a new reaction tube with 5 µl of the PCR-product at room temperature (RT). When handling up to 12 samples it is recommended to perform the hybridization mixes in 8x PCR-stripes and to fill twice 6 tubes in a row. This enables to transfer 6 hybridization mixes at once onto the chip using a multichannel pipette as described in step 5.

3. Incubate the hybridization mix for 2 min at 95 °C in a heating block or water bath.  
**Important Note:** Double stranded DNA is denatured when heated to 95°C. This is important for efficient hybridization.

**Attention:** Danger of burn.

4. To prevent heat evaporation, cool down the hybridization mix for 30 s at RT before opening the tubes.

5. Transfer 25 µl of the hybridization mix into each well of the chip using 6 channels of an 8-channel multipipette.  
**Important Note:** Avoid formation of air bubbles!

6. Incubate the chip for 10 min at 60 °C in a steam-saturated atmosphere.

7. Wash the chip for 10 s in preheated washing buffer I at 60 °C. For this take the chip at its writing area and immerse it into the washing buffer. Verify the temperature of the washing buffers I and II regularly!  
**Important Note:** Avoid the chip surface to become dry!

8. Wash the chip for 20 s in washing buffer II at 60 °C.

9. Wash the chip in washing buffer III at RT (10 s – refer to fig. 4).

10. Remove any liquid from the chip surface by using compressed air or nitrogen.

The ParoCheck™ chip is now ready to be read out, but may also be stored for some days in the dark at RT.

---

**Fig. 4: Hybridization and washing steps**

1. **preheating** 60 °C, > 5 min
2. **add hybridization mix**
3. **hybridization** 60 °C, 10 min
4. **1st wash** 60 °C, 10 s
5. **2nd wash** 60 °C, 20 s
6. **3rd wash** RT, 10 s

---

**4 Data analysis**

Scanning and data analysis using the CheckScanner™ and the CheckReport™ software are described in detail elsewhere. Briefly, the diagnostic software CheckReport™ consists of three independent programs: CheckReport™ SampleSheet, CheckReport™ Results and CheckReport™ Admin. In combination, these modules address all needs of biochip evaluation and data administration required for a diagnostic set-up. The software enables
the fully automated quantification and quality control of ParoCheck<sup>®</sup> DNA-chips. Working with the Check Report suite generally consists of the following steps:

- Use CheckReport™ Sample Sheet to assign patient samples to Chips or Strips and to print out the pipetting scheme for the assay proper
- Perform the assay
- Use CheckReport™ Results to scan and analyze the chip and to print automatically generated reports.
- Use CheckReport™ Admin. You need to have administrator privileges to use this module

The CheckReport™ suite supports the use of the microarray CheckScanner™, which allows to scan up to four chips in a row. The module CheckReport™ Results enables to start the CheckScanner™ as well as analyzing existing images or importing images generated by other scanner types.

In combination, the CheckScanner™ and the CheckReport™ suite address all needs of biochip evaluation and data administration required for a diagnostic chip.

**Important Notes for the reliable use of CheckReport™ software:**

Whenever analysing data using CheckReport™ Results make sure that:

- the plug-in version installed on your PC corresponds to the one mentioned on the kit. If not, reinstall the revised version.
- the right image respectively samplesheet is loaded. Information about image and samplesheet are displayed on the report.
- you control the success of automated spot finding for each well. In case of dust on the chip, spots may not be characterized correctly. If so, adjust the spot diameter manually as described in the Check Report User Guide.

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### 5 Performance data of the ParoCheck<sup>®</sup> 20 DNA chip

#### 5.1 Limit of detection (LOD)

The LOD of ParoCheck<sup>®</sup> was determined by multiple analysis of serial dilutions of bacterial DNA under presence of sample matrix. The LOD for each bacteria was calculated for a cut off value of the signal to noise ratio equal 12.

**Table 3:** LOD for 20 bacteria analyzed with ParoCheck<sup>®</sup> 20

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>LOD* for cut of ≤ 12</th>
<th>Bacterium</th>
<th>LOD* for cut of ≤ 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. actinomycetemcomitans</td>
<td>617</td>
<td>A. odontolyticus</td>
<td>120</td>
</tr>
<tr>
<td>A. viscosus</td>
<td>6013</td>
<td>C. gingivalis</td>
<td>435</td>
</tr>
<tr>
<td>T. forsythensis</td>
<td>935</td>
<td>C. concisus</td>
<td>650</td>
</tr>
<tr>
<td>C. rectus</td>
<td>1433</td>
<td>C. gracilis</td>
<td>1721</td>
</tr>
<tr>
<td>T. denticola</td>
<td>2062</td>
<td>E. nodatum</td>
<td>369</td>
</tr>
<tr>
<td>E. corrodens</td>
<td>787</td>
<td>P. nigrescens</td>
<td>945</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>540</td>
<td>S. constellatus</td>
<td>1145</td>
</tr>
<tr>
<td>P. micros</td>
<td>1148</td>
<td>S. gordonii</td>
<td>490</td>
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<tr>
<td>P. gingivalis</td>
<td>586</td>
<td>S. mitis</td>
<td>473</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>637</td>
<td>V. parvula</td>
<td>159</td>
</tr>
</tbody>
</table>

*copies of 16S-rRNA-Gen per PCR

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### 6 Technical Support

How to contact us:

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Email: biochips@gbo.com  | Internet: www.gbo.com/bioscience
7 Control of results and troubleshooting guide

<table>
<thead>
<tr>
<th>Result after Reading</th>
<th>Possible Error and Causes</th>
<th>Possible Controls and Checks</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>No spots detectable, no orientation points detectable</td>
<td>• Chip has been inserted incorrectly&lt;br&gt; • Chip is damaged or defective&lt;br&gt; • Scanner is defective</td>
<td>• Check the chip orientation in the scanner&lt;br&gt; • Check the scanner program</td>
<td>• Scan an unused chip to control the correct settings of scan parameters</td>
</tr>
<tr>
<td>Signals detected from the orientation spots only</td>
<td>• No or incorrect hybridization&lt;br&gt; • Incorrect dilution of washing buffer</td>
<td>• Check the hybridization temperature&lt;br&gt; • Check the solutions for washing and hybridization</td>
<td>• Re-assay with new hybridization solution</td>
</tr>
<tr>
<td>Orientation and hybridization controls</td>
<td>No or weak PCR reaction&lt;br&gt; PCR inhibitors&lt;br&gt; No amplified product was mixed with the hybridization solution&lt;br&gt; No amplification of PCR control template due to very high amounts of bacterial DNA (competition). Results may be o.k.&lt;br&gt; Incorrect or too long storage of hybridized chips</td>
<td>• Check your PCR protocol&lt;br&gt; • Check your PCR instrument and program&lt;br&gt; • Check the DNA. Probably dilute DNA before PCR&lt;br&gt; • Check for inhibitors&lt;br&gt; • Check the PCR product by DNA gel electrophoresis (ca.300bp)</td>
<td>• Perform the protocol without DNA samples – only the PCR control should be positive</td>
</tr>
<tr>
<td>PCR control shows a weak signal, SNR (signal-to-noise ratio)</td>
<td>PCR inhibitor&lt;br&gt; Weak amplification of PCR control template due to very high amounts of bacterial DNA (competition). Results may be o.k.&lt;br&gt; Incorrect or too long storage of hybridized chips</td>
<td>• Check the PCR product by DNA gel electrophoresis (ca.300bp)</td>
<td>• Perform the protocol without DNA samples – only the PCR control should be positive</td>
</tr>
<tr>
<td>High number of positive signals by the pathogenic spots</td>
<td>Possible true result&lt;br&gt; Hybridization temperature too low;&lt;br&gt; Incorrect performance of the washing protocol;&lt;br&gt; Use of incorrect washing buffer</td>
<td>• Check the red signals of the hybridization control spots, they should come to 0&lt;br&gt; • Check the washing and hybridization conditions</td>
<td>• Repeat the washing procedure and read out the chip again&lt;br&gt; • Analyze a known sample or a negative control</td>
</tr>
<tr>
<td>No bacteria detected</td>
<td>Possible true result</td>
<td>• If the result is called in question, make sure that DNA and polymerase were added to the PCR Mix</td>
<td>• Repeat the PCR and hybridization and make sure that all components according to 3.5. are added to the reaction</td>
</tr>
</tbody>
</table>

8 References

8.1 References to ParoCheck®

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