Mentype® **MycoDerm**QS

Lateral Flow

Modular multiplex polymerase chain reaction (PCR) for a fast and reliable diagnosis of dermatomycosis pathogens

Instructions for use

CE IVD

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Made in Germany

Biotype Diagnostic GmbH develops molecular biological in-vitro diagnostics (IVD) for haematology/oncology, dermatology and urology. The company has many years of experience in developing and producing DNA analysis test systems. Biotype works with scientific experts to produce innovative product ideas and high quality, customer-oriented applications which meet the demands of the diagnostics sector. Our Mentype® product ranges represent the highest quality in clinic diagnostics.

For more information and enquiries about Mentype® **MycoDerm**^{QS} Lateral Flow please contact us: www.biotype.de.

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Trademarks and patents

Mentype® is a registered trademark of Biotype Diagnostic GmbH.
Mastercycler® is a registered trademark of Eppendorf AG.
QIAamp® is a registered trademark of Qiagen GmbH.

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Contents

1. Product description

Mentype® MycoDermQS Lateral Flow is a molecular biology diagnostic tool, used to efficiently diagnose dermatomycoses (based on skin, hair and nail samples) in a medical laboratory. The amplification of specific DNA sequences, which are located in the "marker gene areas" of each dermatomycosis pathogen, allows a species specific differentiation.

Mentype® MycoDermQS Lateral Flow is a modular assay. Three different multiplex PCR approaches can be used and combined as required. The final evaluation of the specific DNA sequences (meeting the requirements of RiliBÄK and MiQ) is carried out by using universal Lateral Flow stripes.

- **Mentype® MycoDerm**QS Lateral Flow **PCR 1**
  Detection of: dermatophytes (class), yeasts (class), *Trichophyton rubrum* and *Scopulariopsis brevicaulis*

- **Mentype® MycoDerm**QS Lateral Flow **PCR 2**
  Detection of dermatophytes: *T. tonsurans , T. violaceum,.* Detected as a cluster: *T. interdigitale* and *T. mentagrophytes* as well as *T. benhamiae/T. erinacei.* and *T. verrucosum*

- **Mentype® MycoDerm**QS Lateral Flow **PCR 3**
  Detection of dermatophytes: *M. canis*, *Epidermophyton floccosum, Nannizzia gypsea* as well as *M. audouinii*.

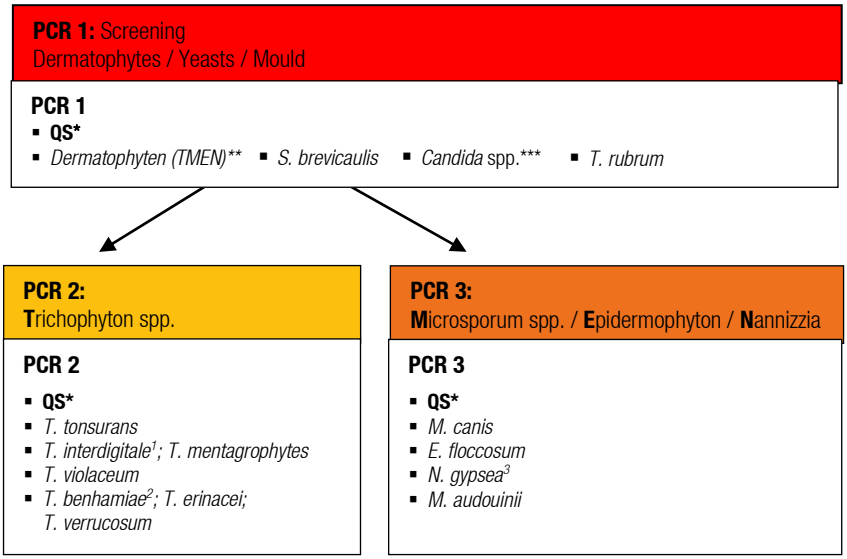
The assay enables the detection of 19 relevant dermatophytes, yeasts and moulds common Europe [1], nine of them species-specific.

The selection and differentiation of the pathogens (see scheme 1) were made in consultation with clinical experts and contribute to the fast diagnosis of the cause of illness as well as to situation-specific treatment decisions.

In accordance with the microbiology and infectiological diagnostics quality standards (MiQ) of the Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM) [2] and the RiLiBÄK 2015 the Mentype® **MycoDerm**QS Lateral Flow includes an internal extraction and PCR amplification control (quality sensor, QS). If a negative pathogen is detected (missing pathogen amplificate), this serves as a confirmation that the PCR has been successfully carried out. The required detection

The kit also include hybridisation probes to enable the quick and secure classification of the pathogen amplificates.

Scheme 1:
Modular detection of dermatomycosis pathogens by **Mentype® MycoDerm^{QS} Lateral Flow** (overview)



* **QS** = DNA extraction and PCR amplification control according to RiLiBÄK and MiQ

** **TMEN** also displays the following types:
T. terrestre (geophilic): - *Arthroderma insingulare*
- *Arthroderma quadrifidum*
- *Arthroderma lenticulare*
Microsporum ferrugineum

*** *Candida* spp. genus specific types:
C. albicans
C. tropicalis
C. glabrata
C. krusei (Pichia kudriavzevii, Issatchenkia orientalis)
C. guilliermondii (Meyerozyma guilliermondii)
C. parapsilosis

[1-3] Formerly: [1]*T.interdigitale/A.vanbreusegehemii,* [2]T.Species von *A. benhamiae*, [3]*M. gypseum.* Neue Nomenklatur nach: Hoog, G.S., Dukik, K., Monod, M. et al. Mycopathologia (2017) 182: 5. doi:10.1007/s11046-016-0073-9

Kit contents

Content of the different Mentype® **MycoDerm**QS Lateral Flow PCR amplification kits for PCR 1, PCR 2, PCR 3 and PCR 2&3
Number of reactions: 50 each

Kit components	PCR 1	PCR 2	PCR 3	PCR 2&3
Nuclease-free Water	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Reaction Mix **A** (REM **A**)	500 µl	500 µl	500 µl	500 µl
Multi Taq2 DNA-Polymerase	25 µl	25 µl	25 µl	25 µl
LF Primer Mix **PCR 1**	50 µl			
LF Primer Mix **PCR 2**		50 µl		25 µl
LF Primer Mix **PCR 3**			50 µl	25 µl
LF Control DNA **PCR 1**	10 µl			
LF Control DNA **PCR 2**		10 µl		10 µl
LF Control DNA **PCR 3**			10 µl	10 µl
LF Quality Sensor (QS)	800 µl	800 µl	800 µl	800 µl
LF Hybrid Mix **PCR 1**	10 µl			
LF Hybrid Mix **PCR 2**		10 µl		10 µl
LF Hybrid Mix **PCR 3**			10 µl	10 µl
LF Running Buffer	5.5 ml	5.5 ml	5.5 ml	5.5 ml
Lateral Flow Stripes	50 piece	50 piece	50 piece	50 piece
Buffer **L** (conc.)*	3x 0.6 ml	3x 0.6 ml	3x 0.6 ml	3x 0.6 ml
Buffer **N**	2x 2 ml	2x 2 ml	2x 2 ml	2x 2 ml

* Re-suspend the tube contents in a volume of 1.4 ml nuclease-free water.
(Do not use the nuclease-free water that is provided in the kit.)

Storage

- The PCR reagents (REM A, Taq, primer, H_20, probes) as well as the standards (control DNA and quality sensor (QS)) should be stored at -20 °C. Repeated thawing and freezing should be avoided.
- The control DNA must be stored separately from the PCR reagents (separation of the areas pre and post PCR to avoid contamination).
- The Lateral Flow stripees, LF running buffer and buffer **L** and **N** can be stored at room temperature. If the LF running buffer is not used for a longer period of time, it can stored at 2 to 8 °C.
- The expiry date is indicated on the kit cover.

Area of application

The Mentype® **MycoDerm**QS Lateral Flow kit is a fully-fledged in vitro diagnostic (IVD) for detecting dermatophytes, yeasts and non-dermatophyte moulds in clinical samples of suspected dermatomycosis. Nail material and dandruff provide suitable sample material, as

do hair fragments. The PCRs display ideal results when 10 pg to 400 pg of pathogen DNA is used.

Extensive validations, including a clinical performance evaluation in accordance with the requirements of the In-Vitro Diagnostics Directive 98/79/EC (IVDD) have been performed. A declaration of conformity has been issued and the kit has been registered with the appropriate authorities as a CE-IVD under DE/CA83/2012-39.

This product is designed to be used by qualified personnel (e.g. lab technicians and doctors, who have been trained in molecular biology techniques). All diagnostic results, produced by the application of the sample preparation process in conjunction with a diagnostic test must be analysed under consideration of other clinical findings or lab results for a complete anamnesis.

Before starting your work please read the Instructions for Use carefully and follow the instructions. Integrate the prozesses as described in your quality management and validate it within your laboratory.

To keep deviations in the diagnostic results as low as possible, suitable controls have to be carried out.

Safety information

Always wear a lab coat, protective gloves and goggles when handling chemicals. Safety data sheets for all kit components can be requested from Biotype Diagnostic GmbH. Please contact the respective manufacturer for safety data sheets of reagents which are not part of this test kit. Ensure a correct disposal of sample material and kit components.

This test kit contains the following potentially hazardous substances:

Kit component	Chemical	Percentage	Hazards (H) and precautions (P)
Buffer **L** (conc.)	KOH	10 %	**GHS05: Corrosion risk** // H302 (harmful if swallowed), H314 (causes severe skin burns and eye damage), **GHS07 Caution!** // H290 (can be corrosive to metals) P280 (wear protective gloves / protective clothing / eye protection / face protection), P301+309+310 (if swallowed, rinse mouth with water; do not induce vomiting), P305+351+338 (if contact with the eyes occurs, carefully rinse the eyes with water for several minutes; remove contact lenses if possible, continue to rinse eyes), P309+P310 (in the event of exposure or if you feel unwell, call a poison control centre or a doctor)

Quality assurance

The quality of the test kits is continually inspected against pre-set specifications, to demonstrate their unlimited usability.

For any questions about quality assurance please contact us at support@biotype.de.

Disclaimer

Please note that confusion in samples and/or contaminations as well as the incorrect use or application of this product and the use of Lateral Flow readers, which are not validated by the Biotype Diagnostic GmbH, can result in wrong diagnosis. A systemic treatment, based on incorrect findings can lead to life-threatening damage under certain circumstances. The Biotype Diagnostic GmbH does not accept any liability for accidental or sequential damage of any kind that arise from failure to comply with this instruction.

2. Overview workflow

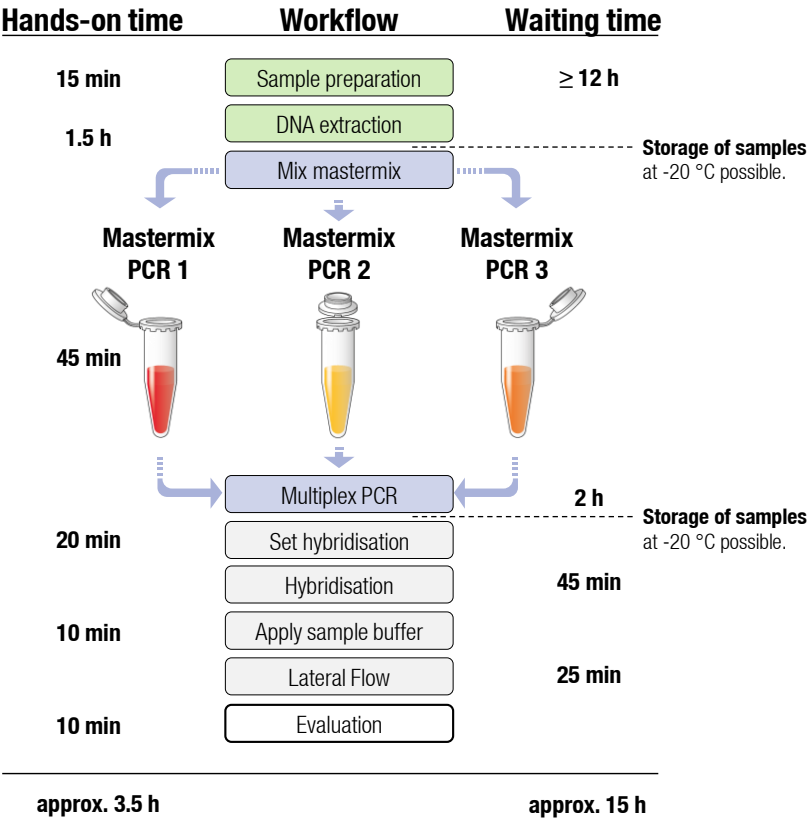


Illustration 1: From sample to detection - diagnosing dermatomycosis with the help of Mentype® **MycoDerm**^{QS} Lateral Flow PCR amplification kits. (time refers to a formulation with 20 samples).

3. Equipment to be provided by the user and additional reagents needed

For lab work:

- Lab protective clothing (lab coat, protective gloves and goggles)

For sample preparation and amplification:

- Pipettes* and pipette tips (to avoid cross contamination we recommend using pipette tips with aerosol barriers)
- Disposable gloves (powder-less)
- Thermoblock* for the lysis of the samples (e.g. the Eppendorf® Thermomixer comfort with a thermoblock for 2 ml micro reaction vessels)#
- Micro and mini centrifuge*
- Vortexer
- Thermocycler with a heating rate of at least 4 - 6 °C#
- Nuclease-free water

The following reagents will also be needed:

Reagent	Provider/supplier#	Order number
QIAamp® DNA Mini Kit	Qiagen GmbH	51304
Ethanol 96 - 100 %	Applichem GmbH	A1868,1000

*To ensure the samples are prepared properly using the kit, we recommend calibrating the devices (e.g. pipettes and thermoblocks) in accordance with the manufacturer's recommendations.
#This is not a complete supplier list; many important lab equipment distributors are not listed.

4. Important pre-preparation information

- Check the kit components for damage after receipt.
- If the buffer containers are damaged, please contact Biotype Diagnostic GmbH's technical department or your supplier. If any fluids have leaked, please consult the "Safety Information" section (page 9). Do not use any damaged kit components, as this could affect the kit's efficiency.
- Change the pipette tip after each pipette work step. To protect against cross contamination we recommend using pipette tips with aerosol barriers.
- All centrifuge steps should be carried out at room temperature (15 - 25 °C).
- Always wear disposable gloves and regularly check that they are not contaminated with sample material (change gloves if necessary).
- Dispose of the gloves if they are contaminated.
- Always open only one container at a time, to avoid cross contamination.
- Do not combine components from different kits, unless the lot numbers are identical.
- Avoid the microbiological contamination of the kit reagents.
- To keep the risk of an infection from potentially infectious material as low as possible, we recommend working under sterile bench conditions right up to the lysis of the samples.
- This kit should only be used by personnel who have been trained in the processes of in vitro lab diagnostics and the PCR methodology.

5. Protocols for DNA extraction

Important note!
Depending on the condition of the patient samples two different protocols are recommended:

- **Lysis of skin, hair and nail clipping samples** see chapter **5.1.1**
- **Lysis of complete nails** see chapter **5.1.2**.

5.1 Lysis of patient samples with subsequent DNA isolation using DNA-free standard centrifuge tubes (1.5 ml)

To isolate and purify DNA from skin, hair, nail clippings and complete nail samples, DNA isolating kits which use silica membrane technology should always be used. Sample lysis and DNA extraction should be done in accordance with the instructions and recommendations of the relevant manufacturer.

Note:

1. To ensure optimal tissue disintegration, we generally recommend **incubation with proteinase K overnight** or for at least 12 hours.
2. During the elution procedure the **volume of the elution buffer** should be reduced from 200 µl to **50 µl**.

5.1.1 Lysis of skin, hair and nail clippings samples with subsequent DNA isolation

The following describes the lysis of the samples and the DNA isolation using Qiagen QIAamp® DNA Mini Kits.

To prepare the **lysis buffer, 180 µl ATL buffer** and **20 µl Proteinase K** per sample are mixed. Per formulation, a 5 % excess is calculated, to avoid pipetting errors. The resulting buffer can be used further immediately.

To examine and ensure a contamination-free workflow the addition of a **process control** is recommended. This control do not contain any sample material (e.g. swab), should be treated like a sample and processed together with the other samples.

During the DNA extraction the **Quality Sensor (QS)** must be added **to each sample** (including the process control) (see protocol).

1. **200 µl of lysis buffer** (180 µl ATL* + 20 µl Proteinase K) is added to the sample material in the standard centrifuge tube (1.5 ml) and the lid should be sealed.

* Buffer precipitates at low temperatures. To dissolve the buffer, shortly incubate it at 56 °C. Add it to the sample afterwards.

2. Add **15 µl of Quality Sensor (QS)** to each sample and seal the lid.
3. Incubat overnight or for at least **12 hours** in a thermomixer at **56 °C** and **850 U/min**.
4. Centrifuge for **1 min** at **8,000** to remove condensation from the lid.
5. Add **200 µl of buffer AL**, seal the lid and mix (vortex **15 s**).
6. Incubate at **70 °C** and **850 U/min** for **10 min** in a thermomixer.
7. Centrifuge for **1 min** at **8,000** to remove condensation from the lid.
8. Add **200 µl of ethanol (96 %)**, vortex **15 s** and centrifuge briefly.
9. Transfer the supernatant (**600 µl lysate without any pellet**) and severed swab into **QIAamp® Mini Kit column** and centrifuge at **8,000 U/min** for **1 min**. Remove the swab afterwards and discard the collection tube and flow-through.
10. Place the QIAamp® Mini Kit column in a new collection tube.
11. Add **500 µl of buffer AW1** and centrifuge at **8,000 U/min** for **1 min**. Discard the collection tube and the flow-through.
12. Place the QIAamp® Mini Kit column in a new collection tube.
13. Add **500 µl of buffer AW2** and centrifuge at **13,300 U/min** for **3 min**. Discard the collection tube and the flow-through.
14. Place the QIAamp® Mini Kit column in a new collection tube.
15. Repeat centrifugation at **13,300 U/min** for **1 min**.
16. Place the QIAamp® Mini Kit column in a new, DNA-free standard centrifuge tube (1.5 ml) and discard the collection tube.
17. Add **50 µl of buffer AE** and incubate for **1 min** at room temperature.
18. Centrifugation at **8,000 U/min** for **1 min** to elute the DNA and discard the QIAamp® Mini Kit column.

The eluted DNA isolate (in 50 µl buffer AE) can directly be used for PCR amplification or stored at -20 °C (see **chapter 5.2**).

5.1.2 Lysis of complete nails with subsequent DNA isolation

For the effective reprocessing of nail samples (large/complete nails, especially thick toenails) we recommend using buffer **L** and buffer **N**, which are included in the Mentype® **MycoDerm**QS Lateral Flow kit.

The following describes the lysis of the samples and the DNA isolation using Qiagen QIAamp® DNA Mini Kits.

Before being used for the first time, the concentrate of the buffer **L** should be adjusted with 1.4 ml of nuclease-free water (not included in kit) to produce the usage concentration. The buffers **L** and **N** should be used at room temperature.
Here the first kit-specific buffer (ATL) of the DNA isolating kit is replaced by buffer **L**.

To examine and ensure a contamination-free workflow an additional **process control** is recommended. This control does not contain any sample material, but has to be treated like any other sample and processed together with the other samples.

During the DNA extraction the **Quality Sensor (QS)** must be added **to each sample** (including the process control) (see protocol).

1. **100 µl of buffer L** (Mentype® **MycoDerm**QS Lateral Flow; adjusted to usage concentration, **room temperature**) is added to the sample material in the standard centrifuge tube (1.5 ml) and the lid should be sealed.

2. Incubate at **90 °C** and **850 U/min** for **15 min** in a thermomixer.

3. Cool the sample to **56 °C** and centrifuge for **1 min** at **8,000 U/min** to remove condensation from the lid.

4. Add **80 µl of buffer N** (Mentype® **MycoDerm**QS Lateral Flow; **room temperature**) and **20 µl Proteinase K**, close the lid and mix (vortex).

5. Add **15 µl of Quality Sensor (QS)** to each sample and seal the lid.

Further DNA extraction and purification is carried out analogously to **chapter 5.1.1 working step 3** (incubation overnight).

5.2 PCR amplification

5.2.1 Formulation of the master mix for PCR 1, PCR 2 and PCR 3

All reagents has to be kept at room temperature for the shortest possible time and immediately stored again at -20 °C after use. Cool racks keep the samples cool during sample preparation (note temperature-indicating colour change of cool racks). Before use, the reagents or master mixes should be mixed well (vortexed) and briefly centrifuged (5 s).

- Add the recommended sample volume (1 µl template DNA, resulting from DNA extraction in chapter 5.1 ff/ 6.1 ff) to the master mix (a total reaction volume of 25 µl should be reached).
- Take into account the negative controls of **PCR 1, PCR 2** and **PCR 3** when calculating the number of PCR reactions. Add one or two reactions to this number, in order to compensate for pipette errors.

PCR Master mix

The following table shows the volumes of the kit components used for the formulation of a single master mix of **PCR 1, 2** and **3:**

Component	Volumes
Nuclease-free Water	17.5 µl
Reaction Mix **A***	5.0 µl
Primer Mix (PCR 1, 2 or 3)	1.0 µl
Multi Taq2 DNA-Polymerase (hot start, 2,5 U/µl)	0.5 µl
Volume of the master mix	**24.0 µl**

*contains Mg^{2+}, dNTPs, BSA

Note:

The **PCRs** display ideal results when 10 pg to 400 pg of pathogen DNA is used. For reference material and isolates of culture plates, the use of 1 µl of DNA isolate (dilute to 250 or 100 pg/µl) is usually sufficient.

For patient material we recommend using optimal volumes of 1 µl of DNA isolate. DNA isolates extracted from nails of treated patients should be diluted (1:4 with buffer TE).

When using other DNA sample volumes, the volumes must be adapted as needed with nuclease-free water, so that the volumes of the PCR formulations come to 25 µl.

5.2.2 Positive controls

To ensure the maximum sensitivity of the assay, the addition of a low concentration positive control in both PCR formulations (**PCR 1, PCR 2** and **PCR 3**) is recommended.

- For this, dilute the **control DNA PCR 1, 2 and 3** by dilution factor **1:20 in buffer 1x TE.**
- Pipette **1 µl of diluted control DNA** instead of the template DNA into the reaction vessel with the PCR master mix.

5.2.3 Negative controls

For the negative control (= process control), pipette nuclease-free water instead of the template DNA into the reaction vessel with the PCR master mix for the **PCR 1, PCR 2** or **PCR 3** formulations. If no process control was prepared, a negative control has to be used.

- For this, dilute the **Quality Sensor (QS)** by dilution factor **1:20 in buffer 1x TE.**
- Pipette **1 µl of diluted Quality Sensor (QS)** into the reaction vessel with the PCR master mix.

In both cases, after amplification and hybridisation, no further amplificates should be detected on the LF stripe apart from the QS band (see illustration 3).

5.2.4 PCR amplification parameters

The **PCR 1/ PCR 2/ PCR 3** formulations are amplified under the same PCR conditions at the same time. To activate the Multi Taq2 DNA-Polymerase and to suppress the formation of unspecific amplification products, the carrying out of a "**hot start**" is highly recommended.

PCR parameter 1:

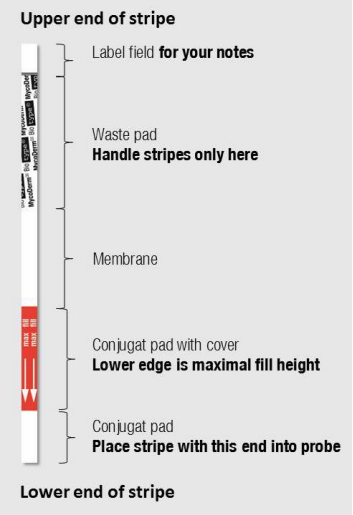
Temperatur*	Zeit*	
96 °C	4 min (hot start for the activation of the Multi Taq2 DNA Polymerase)	
96 °C	30 s	
60 °C	60 s	**35 cycles**
72 °C	60 s	
10 °C	∞	until the end

*Parameter of amplification for thermocycler with silver or aluminium-coated blocks.

Adjust the **heating rate** (ramping rate) of the thermocycler at **3 °C/s** prior to amplification.

Briefly centrifuge after PCR amplification to remove condensation. The validation of the test kit has been done with the Mastercycler® ep gradient S (Eppendorf AG).

5.3 Lateral Flow



The evaluation of the PCR amplificates is carried out by Lateral Flow stripes. To each PCR approach the specific probe mix is applied and then hybridized in the PCR thermocycler. Afterwards a part of the hybridisation mix is added to the LF running buffer. The LF stripes are placed in the final hybridisation mixture. The mix is absorbed by the LF stripes and results in a specific dyeing of the corresponding pathogenic and control band.

Illustration 2: Structure of Lateral Flow test stripe and instructions for handling

Note:

The LF stripes are universal applicable for each of the three test modules. A unmistakable labeling of the LF stripes before hybridisation is recommended to avoid confusion after hybridisation (e.g. sample and PCR). The establishment of a color code for every PCR is another possibility. Commercially colored labels can be used. Label and stick them on the upper end of the stripe (label field).

5.3.1 Dilution and activation of LF Hybrid Mixes

Dilute the provided probe mix 1:8 in 1x TE buffer. When calculating the required quantity consider the number of hybridisation mixes to be processed. The tube should contain a minimum volumne of 10 µl. Activate the diluted LF Hybrid Mix with the following PCR conditions:

PCR parameter 2:

Temperature	Time
98 °C	10 min
25 °C	5 min
10 °C	∞

Briefly centrifuge after activation to remove condensation.

Adjust the **heating rate** (ramping rate) of the thermocycler at **3 °C/s** prior to activation of LF Hybrid Mixes.

5.3.2 Hybridisation

Preparation of the hybridisation mixes:

1. Apply 1 µl of the diluted and activated LF Hybrid mix to the corresponding PCR apporach (e.g. 1 µl diluted LF Hybrid mix **PCR 1** to 25 µl **PCR 1** mix).
2. Briefly vortex the hybridisation mix
3. and briefly centrifuge
4. Hybridisation in the PCR cycler under the following conditions:

PCR parameter 3:

Temperature	Time	
98 °C	10 min	
65 °C	20 min	
10 °C	∞	max. 15 min!

Briefly centrifuge after hybridisation to remove condensation.

Notes:

Adjust the **heating rate** (ramping rate) of the thermocycler at **3 °C/s** prior to hybridisation.

The cooling step **should not take more than 15 min**. Otherwise the hybridisation products could fall into pieces again.

Use only freshly activated LF Hybrid Mixes for the preparation of the hybridisation mixes. Discard excess amounts of activated LF Hybrid Mixes.

5.3.3 Lateral Flow

1. Add 100 µl LF running buffer in a new sample tube (1.5 ml)
2. Add 10 µl hybridisation mix to 100 µl LF running buffer tube
3. Vortex and centrifugate briefly
4. Hold the LF stripe at the upper end (writing "Biotype / **MycoDerm**^{QS}")
5. Place the LF stripe with the lower end (white arrow down) in the hybridisation mixture
6. Incubate at room temperature for 25 min
7. Evaluate the LF stripe

Note:

After 2 hours the LF test stripes are completely dry and can be archived. The intensity of the bands can decrease over time.

5.4 Evaluation

A successful experiment is characterised by a visible Quality Sensor (QS) band on every LF stripe. This band serves as a DNA extraction control, amplification control and inhibition control for the PCR as well as functionality control of the LF stripes.

The validity of the test is guaranteed when the process control does not show any additional band to the QS and the positive controls for each PCR have been performed successfully (illustration 3).

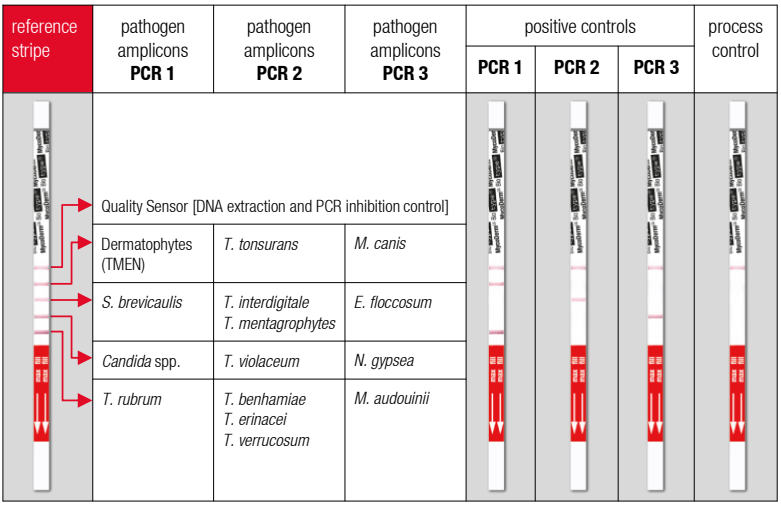


Illustration 3: Lateral Flow stripes with the typical band patterns of the process control and the positive controls from each PCR evaluation template (not in original size).

Illustration 3 depicts the evaluation template with a reference stripe (not in original size). The reference stripe serves as reference for the evaluation of the pathogenic amplificates from **PCR 1**, **2** and **3**. With the help of this stripe the amplificates can easily be assigned to the corresponding pathogens for every PCR. The band on top of the LF stripe is always the Quality Sensor (QS).

6. Alternative protocols for lysis of patient samples with subsequent DNA isolation

Depending on the condition of the patient samples two different protocols are recommended:

- **Lysis of skin, hair and nail clipping samples** see chapter **5.1.1**
- **Lysis of complete nails** see chapter **5.1.2**.

6.1 Lysis of patient samples with subsequent DNA isolation using Sampletype i-sep® SQ

To isolate and purify DNA from skin, hair, nail clippings and complete nail samples, DNA isolating kits which use silica membrane technology should always be used and done in accordance with the instructions and recommendations of the relevant manufacturer.

Note:

1. **During the DNA extraction** the **Quality Sensor (QS)** must be added **to each sample** (including the process control) (see protocol).
2. To ensure optimal tissue disintegration, we generally recommend **incubation with proteinase K overnight** or for at least 12 hours.
3. During the elution procedure the **volume of the elution buffer** should be reduced from 200 µl to **50 µl**.

6.1.1 Lysis of skin, hair and nail clippings samples with with subsequent DNA isolation using Sampletype i-sep® SQ

The following describes the lysis of the samples and the DNA isolation using Qiagen QIAamp® DNA Mini Kits.

To prepare the **lysis buffer, 180 µl ATL buffer** and **20 µl Proteinase K** per sample are mixed. Per formulation, a 5 % excess is calculated, to avoid pipetting errors. The resulting buffer can be used further immediately.

To examine and ensure a contamination-free workflow the addition of a **process control** is recommended. This control do not contain any sample material (e.g. swab), should be treated like a sample and processed together with the other samples.

During the DNA extraction the **Quality Sensor (QS)** must be added **to each sample** (including the process control).

1. Add **200 µl of lysis buffer** (180 µl ATL* + 20 µl Proteinase K; preheated to **56 °C**) to the sample material in the filter cartridge of the Sampletype **i-sep®** SQ tube and seal the lid.
2. Add **15 µl of Quality Sensor (QS)** to each sample and seal the lid.
3. Incubat overnight or for at least **12 hours** at **56 °C** and **850 U/min** in a thermomixer.
4. Centrifuge for **1 min** at **max. 500 U/min** to remove condensation in the lid.
5. Add **200 µl of buffer AL**, close the lid and mix (vortex **15 s**).
6. Incubate at **70 °C** and **850 U/min** for **10 min** in a thermomixer.
7. Centrifuge for **1 min** at **13,300 U/min**.
8. **Remove the filter cartridge** and close the collection tube.
9. Add **200 µl of ethanol (96 %)**, vortex **15 s** and centrifuge briefly.
10. Transfer the supernatant (**600 µl lysate, without any pellet**) into **QIAamp® Mini Kit column** and centrifuge at **13,300 U/min** for **1 min**. Discard the collection tube and flow-through.
11. Place the QIAamp® Mini Kit column in a new collection tube.
12. Add **500 µl of buffer AW1** and centrifuge at **8,000 U/min** for **1 min**. Discard the collection tube and flow-through.
13. Place the QIAamp® Mini Kit column in a new collecting tube.
14. Add **500 µl of buffer AW2** and centrifuge it at **13,300 U/min** for **3 min**. Discard the collection tube and flow-through.
15. Place the QIAamp® Mini Kit column in a new collection tube.
16. Centrifuge at **13,300 U/min** for **1 min**.
17. Place the QIAamp® Mini Kit column in a new, DNA-free standard centrifuge tube (1.5 ml) and discard the collection tube.
18. Add **50 µl of buffer AE** and incubate for **1 min** at **room temperature**.
19. Centrifuge at **8,000 U/min** for **1 min** to elute the DNA. Discard the QIAamp® Mini Kit column.

The eluted DNA isolate (in 50 µl buffer AE) can directly be used for PCR amplification or stored at -20 °C (see **chapter 5.2**).

* At low temperatures buffer precipitate. The buffer must be heated (56 °C) before adding it to the sample.

6.1.2 Lysis of complete nails with with subsequent DNA isolation using Sampletype i-sep® SQ

For the effective reprocessing of nail samples (large/complete nails, especially thick toenails) we recommend using buffer **L** and buffer **N**, which are included in the Mentype® **MycoDerm**QS Lateral Flow kit.

The following describes the lysis of the samples and the DNA isolation using Qiagen QIAamp® DNA Mini Kits.

Before being used for the first time, the concentrate of the buffer **L** should be adjusted with 1.4 ml of nuclease-free water (not included in kit) to produce the usage concentration. The buffers **L** and **N** should be used at room temperature. Here the first kit-specific buffer (**ATL**) of the DNA isolating kit is replaced by buffer **L**.

To examine and ensure a contamination-free workflow the addition of a **process control** is recommended. This control do not contain any sample material (e.g. swab), should be treated like a sample and processed together with the other samples.

During the DNA extraction the **Quality Sensor (QS)** must be added **to each sample** (including the process control) (see protocol).

1. **100 µl of buffer L** (Mentype® **MycoDerm**QS Lateral Flow; adjusted to usage concentration, **room temperature**) is added to the sample material in the filter cartridge of the Sampletype **i-sep**® Q tube and the lid should be sealed.

2. The sample is incubated in a thermomixer at **90 °C** and at **850 U/min** for **15 min**.

3. Cooling the sample material to **56 °C** and remove condensation in the lid using centrifugation for **1 min** at **max. 500 U/min**.

4. Add **80 µl of buffer N** (Mentype® **MycoDerm**QS Lateral Flow; **room temperature**) and **20 µl Proteinase K**, close the lid and mix (vortex).

5. Add **15 µl of Quality Sensor (QS)** to each sample and seal the lid.

Further DNA extraction and purification is carried out analogously to **chapter 5.1.1 working step 3** (incubation overnight).

7. Troubleshooting

7.1 PCR amplification

Usage of a thermocycler with an uncoated aluminium block:

If the PCR band and/or the band of the QS is missing, please check the block of your thermocycler. Please use the following PCR parameter, when your thermocycler is equipped with an uncoated aluminium block:

Temperature	Time	
96 °C	4 min	
	(**hot start** for activation of the Multi Taq2 DNA Polymerase)	
96 °C	30 s	
60 °C	120 s	**35 cycles**
72 °C	75 s	
10 °C	∞	until the end

For carrying out the PCR amplification a **heating rate** (ramping) of the thermocycler at **3 °C/s** should be used.

PCR product present, QS band suppressed or missing entirely:

In an unfavourable concentration ratio of the QS and the pathogen DNA, the Quality Sensor's band can be suppressed due to a too-high concentration of pathogen DNA in the PCR. To ensure the validity of the reaction, please repeat the PCR with diluted sample DNA (e.g. 1:4). The QS band should be always present.

Note on process control (negative control):

The process control contains only the visible bands of the Quality Sensor (QS). This band also serves as a DNA extraction control, amplification and inhibition control for the PCR and as a running control to demonstrate the functionality of the lateral flow test strip.

When evaluating the lateral flow stripes, there is a negligible non-homogenous color precipitate, which may appear in a weak form at the level of the *T. rubrum* band. It is not a contamination because the TMEN band is missing. The representation of the process control with the visible bands of the QS and the weak colour precipitation is shown in illustration 4.
The occurrence of a TMEN band without or with a *T. rubrum* band in the case of process control is a contamination. It is also a contamination if you detect a band at Position 2 or 3 after the conjugate pad. The reagents are contaminated. Please initiate the appropriate measures.

Illustration 4: Lateral flow test strips with QS band and weak nonspecific color precipitate.

Reagents are contaminated:

- Always use freshly pipetted reaction mixtures.
- Make sure that the pipettes are not contaminated; they should be regularly cleaned/sterilised.
- Use filter tips to avoid contamination.
- Use a separate pipette set for both the pre and post PCR processing.
- Seal each individual reaction mixture after processing.
- Change your gloves regularly.

Note on evaluation of a T. rubrum- or TMEN-positive PCR 1

1. PCR 1 comprises, at position 4 after the conjugate pad, an additional line for the group-specific detection of dermatophytes in sensu strico *Trichophytum*, *Microsporum*, *Epidermophyton*, and *Nannizzia* (TMEN-Zone).

In the sensitivity range of the observed non-specific *T. rubrum* zone, a positive TMEN zone must also be detected in the presence of the pathogen *T. rubrum*.
The TMEN signal serves as a confirmation test for *T. rubrum*, meaning that the likelihood of a false positive result for this pathogen can be excluded.

When evaluating the lateral flow stripes, please note that the detection of the pathogen *T. rubrum* is **only** positive in conjunction with the TMEN band. The intensity of the TMEN band and the *T. rubrum* band must be similar. Illustration 5 shows the corresponding visual representation of the positive detection of the pathogen *T. rubrum.*

Illustration 5: Lateral flow test strips with the typical positive control band pattern from PCR1 (original stripes)

2. PCR 1 comprises, at Position 4 after the conjugate pad, an additional line for the group-specific detection of dermatophytes in sensu strico *Trichophytum*, *Microsporum*, *Epidermophyton*, and *Nannizzia* (TMEN-Zone).
The TMEN signal serves as a confirmatory test for the group-specific detection of dermatophytes.

The detection of a weak, non-homogeneous color precipitate in the *T. rubrum* zone together with the TMEN band is a negative result for the pathogen *T. rubrum*.

Due to the positive TMEN band, please perform the Mentype® **MycoDerm**^{QS} Lateral Flow PCR2 and three tests.

Illustration 6: Lateral Flow test strips with the Quality Sensor, the TMEN band and a weak, non-homogeneous color precipitate at the first detection line *T. rubrum* (marked in blue) (original strip).

TMEN band present in PCR1, no further exciter bands detected in PCR 2 and PCR 3

If the sample material obtained too low concentration of pathogen DNA, a species-specific detection can not be done. The pathogen DNA concentration is below the limit of detection (100 pg). Please ensure the appropriate sampling and sufficient sample material and repeat the experiment.

No band detectable, QS not present (negative control is also missing):

Set-up errors:

- Check whether the indicated volumes and concentrations have been added to all the reagents, and whether the reagents have been stored correctly.
- Before beginning the PCR, the reagents should be well mixed and centrifuged.
- Repeat the PCR with a new reaction formulation.
- For samples (nails, skin, hair) from treated patients it is recommendable to repeat the PCR with a diluted sample (1:4) to reduce the concentration inhibitors, that might be present.
- Frequent freezing-thawing cycles can have a negative influence of the stability of the reagents.

PCR device error (amplification unsuccessful):

- Check whether the correct PCR programme has been selected or programmed.
- Check that the PCR device work proberly and if every maintenance is carried out according to instructions.

Evaporation of the sample during the PCR (look to see whether the reaction mixture still contains the initial volume after the PCR):

- Lid heating must be activated (105 °C recommended).

LF stripe is defective:

- Check the LF stripes for visual defects (bends, cracks, damaged membrane or otherwise).
- Check the color of the standard drying agent of the LF stripes. A change in color (e.g. pink) indicates that the tubes were not closed carefully and the stripes were stored under high humidity conditions. This can result in loss of function of the LF stripes.

Concentration of human DNA in the sample is too high (background):

- Ensure that mainly fungal material is taken during the sampling process.

Cross reactivity in case of atypical high clinical concentrations caused by different cyclers:
PCR 3:

- A weak false positive band for *Microsporum audouinii* caused by high concentrations of *Microsporum ferrugineum*.

PCR 2:

- A weak false positive band for *T. violaceum* caused by high concentrations of *T. rubrum*.

PCR 1:

- A weak false positive band for *Candida* spp. caused by very high concentrations of *Fusarium* spp./*Penicillium* spp. or *Malassezia* spp./*Alternatia mali*.

Cross reactivity pathogens of PCR 1 belong to the commensal accompanying flora of the human skin surface or are saprophytic germs, which do not encounter in such high concentrations, when the samples are collected appropriate.

7.2 Notes on sampling

The samples are generally taken under sterile conditions. Appropriate health and safety measures should be taken when working with potentially infectious patient samples.

Disinfection: Because non-pathogenic airborne pathogens can be found on skin, nails and hair, the lesion suspected of mycosis should be thoroughly cleaned and disinfected with a swab and 70 % ethanol before the samples are taken (reduction of contaminating accompanying flora).

Material quantities: After drying the disinfected area, sufficient material should be taken, as fungi occur in clusters. Material should always be taken from the growth area of the fungi (border area between mycotic alteration and healthy skin/nail area). In contrast to conventional fungal diagnostics, which calls for the collection of live fungal material, coarser dandruff deposits, crusts, scabs and nail clippings without vital fungal elements are also suitable for the molecular genetic detection of dermatomycosis, as this material also contains a significant quantity of detectable genetic material.

Technique: To remove material from infectious parts of the skin, flakes are removed in the direction of the fungus growth zone with a sterile scalpel, a curette or a sharp spoon.
With onychomycoses, the finer the material, the more successful the yield of fungal-infected DNA will be. Easy to remove, brittle parts should be removed (nails can be cut with scissors if necessary) and discarded. The material (nail clippings) must be taken from the affected areas of the nail plate, where the "infected" tissue meets the "healthy" tissue.
Deeper nail parts close to the nail bed and subungual hyperkeratoses should also be included. For white superficial onychomycoses, the material must be obtained by scraping or cutting the white spots.

When removing hairs, existing pus-filled crusts should be carefully removed and the hairs shortened to a length of about 3 - 5 mm. The cut hair is discarded. Then collect 10 - 20 hair stumps with the epilation tweezers (hair roots must be present!). If necessary, the hair stumps should be "dug out". Areas with conspicuous hairs, i.e. grey, discoloured, dull, whitish coverings or broken hairs should be preferred when taking the samples.
For collecting the material, the lysis and the extraction of particle-free DNA lysates we recommend the sample collection and processing tubes Sampletype **i-sep**® SQ (Biotype Diagnostic GmbH; order number 61-00201-0050 or 63-00201-0050). To do this, the patient sample will be taken with a flocked swab soaked in sterile water and transferred to the filter element of the combi container.

Caution! Please do not use cotton swabs for the sampling process.

8. References

[1] Seebacher C, Bouchara JP, Mignon B. Updates on the epidemiology of dermatophyte infections. *Mycopathologia* 166, 335-352, 2008.

[2] Reischl U, Drosten C, Geißdörfer W, Göbel U, Hoffmann KS, Mauch H, Meyer T, Moter A, von Müller L, Panning M, Rabenau HF, Reiter-Owona I, Roth A, Weitz M. MiQ 1-2011, Nukleinsäure-Amplifikationstechniken. In. Podbielski A, Hermann M, Kniehl E, Mauch H, Rüssmann H (Hrsg.) Mikrobiologisch-infektiologische Qualitätsstandards (MiQ). Im Auftrag der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM). 3. Auflage. Elsevier / Urban & Fischer, München, 2011.

9. Symbols


Manufacturer

Contents sufficient for n reactions


Please follow the instructions for use

Order number

In vitro diagnostics

Specifications of Mentype® Mycoderm^{QS} Lateral Flow PCR amplification kits

A Analytical validation

A a) Test equipment

PCR amplification kit: Mentype® **Mycoderm**^{QS} Lateral Flow PCR amplification kit was used according to instruction for use.

DNA concentration specification: DNA concentrations were determined using the *NanoDrop® ND-1000* (PEQLAB Biotechnologie GmbH, Erlangen) (A_{260})., a UV-VIS Spectrophotometer. The ration of A_{260} / A_{280} is an important quality criterion for DNA. The qulity criterion were between 1,7 und 2,0.

Polymerase chain reaction (PCR): Thermocycler *Eppendorf Mastercycler ep* (Eppendorf AG, Hamburg) was used as Standard-Cycler for Polymerase chain reaction PCR1, PCR2 and PCR3.

Hybridisation: Thermocycler *Eppendorf Mastercycler ep* (Eppendorf AG, Hamburg) was used as Standard-Cycler for Hybridisation.

Lateral Flow: The structure of Lateral Flow test stripes and working advice are described in detail in the instruction for use.

Quality sensor and calibrators: The Quality Sensor (QS) and control DNA for PCR 1, PCR 2 and PCR 3 formulations (positive control) are used according to instruction for use. The PCR amplificates of the positive controls were used to guarantee the detection limit of 10 pg

A b) Analytical Specificity

Objective: The investigations served to exclude false positive results due to cross reactivity with saprophytic germs and human DNA.

Methods: Analytical specificity was tested with the bacterial and fungal strains listed in Appendix 1 at 500 pg of genomic DNA. In addition, 500 pg genomic DNA from *Bos taurus* (bovine), *Canis lupus* familiaris (dog), *Felis catus* (cat) and *Cavia porcellus* (guinea pig) was tested.

DNA from animals was from blood samples that were provided as rest material of veterinary examinations. Human DNA from blood samples were tested at 100 ng per PCR.

Results: Human DNA showed no reactivity up to 100 ng per PCR. Also, no cross-reactivity was observed in any of the tested reference strains and the animal and bacterial accompanying flora. The internal amplification control QS (Quality Sensor) has always been positive.

A c) Analytical Sensitivity

Objective: The investigations were used to determine the analytical detection limit (sensitivity). The testing of DNA used, was from reference strains, accompanying flora and host cells.

Methods: A dilution series of 50 ng, 10 ng, 25 ng, 400 pg, 80 pg, 16 pg and 3.2 pg of genomic DNA was tested in all three PCRs with the respective reference strains in single determination.

Results: For the detection of all pathogens, an analytical sensitivity of at least 10 pg of genomic DNA was achieved, in case of *M. audouinii* at least 50 pg of genomic DNA. At 50 ng genomic DNA of *T. rubrum*, the quality sensor band was suppressed while the specific excitation band was visible. To ensure the validity of the reaction, this PCR should be repeated with diluted sample DNA (eg, 1: 4) so that the QS band is safely displayed. This note is included in the instructions for use of the assay.

A d) Testing of different mixed DNA samples (matrix effects)

Objective: In clinical samples it can be assumed that an excess of human DNA may be present. There is also the possibility of a mixed infection of two different pathogens. Since the PCR is subject to end-product inhibition, the analytical sensitivity and specificity were also tested in the presence of human DNA and a second pathogen.

Methods: All pathogens to be detected were tested in a PCR with 10 pg of fungal genomic DNA mixed with 100 ng of human genomic DNA. Furthermore, fungal DNA mixtures of *T. rubrum* and *C. albicans* were tested with the following variable amounts of DNA (one DNA each in excess), as mainly these two pathogens occur in combination (see Clinical Performance Assessment). In addition, NTCs (no template control) containing 100 ng of human genomic DNA were assayed in all thermocyclers used.

pathogens	DNA amount per PCR approach [pg]								
T. rubrum	1000	500	250	100	50	10	50	50	50
C. albicans	10	10	10	10	10	10	50	100	250

Results: In the presence of 100 ng of human DNA, the internal QS Quality Sensor QS was displayed safely and without product inhibition in all three PCRs on the standard *Eppendorf Mastercycler ep* (Eppendorf AG, Hamburg). The clinical study (see Chapter B) showed that the pathogen-specific DNA detection of *Trichophyton* spp. and *Candida* spp. including a QS band even with an excess of human DNA between 100 ng and up to 10 ug of human DNA safely done. A sensitivity of at least 10 pg of pathogen-specific DNA in the presence of 100 ng of human DNA was achieved.

In the mixtures with pathogen specific DNAs, the detection limit of 10 pg of *C. albicans* DNA was reached with an excess of up to 1 ng of *T. rubrum* DNA. With a mixture of 10 pg *T. rubrum* and *C. albicans* both pathogens are reliably detected. With a surplus of up to 250 pg *C. albicans* DNA, the detection limit of *T. rubrum* DNA was 50 pg. Nonetheless, in the clinical performance assessment exam, two patient samples with mixed infections of *T. rubrum* and *Candida* spp. were detected although the *T. rubrum* DNA concentration was only between 1.6 pg and 11.6 pg of DNA.

Cross-reactivity and amplification inhibition was not found in any of the listed mixtures. The two pathogens were represented safely in mixtures. They were identified in the fungal mixed samples in the above concentrations, all bands including the QS band could be detected.

A e) Testing the influence of different annealing temperatures in the PCR

Objective: To determine the robustness of the PCRs, temperature fluctuations were simulated for the priming annealing step of the PCRs. This temperature step is critical for the sensitivity and specificity of the PCRs. The acceptance criterion was: no interfering signals visible and comparable specific band strength in comparison with the standard reaction and QS always visible.

Methods: Primer annealing temperatures in the PCR were varied by ± 1 ° C and ± 2 ° C (plus -5 ° C) by the kit-specific annealing temperature. The standard reaction was carried out with control DNAs in the nominal concentration (50 pg), each with a single determination with the same PCR master mix, simultaneously in parallel in identical cyclers (Eppendorf Matercycler ep 1 + 2).

Results: PCR 1 showed no visible change in specific band strength at all annealing temperatures used. PCRs 2 and 3 also met the criteria, with *A. benhamiae / T. verrucosum* (PCR 2) at 62 ° C and *M. canis* and *M. audouinii* (PCR 3) were producing weaker signals at 58 ° C. At deviations of ± 1 ° C from the standard annealing temperature, no changes in specific band strength occurred. The QS was shown in all reactions; noise signals did not occur.

A f) Testing the short term stability

Objective: The stability of the reagents of the PCR kit was tested after repeated freezing and thawing.

Methods: The kit reagents were subjected to a 20-fold freeze-thaw cycle. The freezing was carried out for at least 1 h at -20 ° C. Thawed was at room temperature (also at least 1 h) and the reagents were homogenized by shaking before usage. Subsequently, PCRs 1, 2 and 3 were carried out with the respective control DNAs in a final concentration of 10 pg and 50 pg per reaction batch.

Results: The PCR amplificates of all organisms could be detected correctly. The pure QS samples and the entrained NTCs (no template control) contained only the QS band, which was stably displayed. The kit is currently only offered in a pack of 50 reactions (or 10 reactions of the PCR 1 to get to know). Thus, the customer should generally not exceed a 20-fold freeze-thaw cycle. Nevertheless, the labeling recommends to avoid repeated freezing and thawing. The customer may advised to aliquot the reagents.

B Performance evaluation test according to § 24 MPG (Medical Devices Act)

B a) Conditions

A performance evaluation test according to §§ 20 to 24 Medical Devices Act (DE) was carried out. The exemption from the authorization requirement for medical devices with a low safety risk according to § 7 Ordinance on Clinical Trials of Medical Devices was issued by the Federal Institute for Drugs and Medical Devices. An approving vote from the responsible ethics committee and written patient consent declarations were available.

B b) Methods

B b) a) Sampling and Shipping

Sampling is a critical step. It must be assumed that the test material is not homogeneously interspersed with fungal elements.

Sampling was performed by trained hospital staff according to standard procedures of the clinic. Before removing dander, nail shavings and hair shafts, the donor sites were disinfected by using 70 % ethanol. As swabs for PCR analysis sterile FLOQSwabs Copan made of polyurethane foam were used (source: Mast Diagnostics, Reinfeld).Therewith dander from sterile Petri dishes were also taken (swab was previously wetted with sterile water) and transferred to sterile disposable reaction vessels (1.5 ml) before being transported to the examination laboratory.

Attention: When using other sampling swabs, especially from cellulose, the DNA recovery after extraction was worse!

B b) b) Native preparation

Native preparations for bright field microscopy were prepared according to William *et al.* [1] with the following modifications. The material was completely wetted on a microscope slide with Chlorazol E solution (180 mg of chlorazole E, 10 ml of dimethyl sulfoxide, 90 ml of 7.5 % KOH, chemicals from Sigma-Aldrich GmbH, Freiburg), covered with a coverslip and incubated 10 min in a moist chamber at room temperature. Subsequently, the presence of fungal elements was investigated by means of phase contrast microscopy (400x, Axioskop 40, Zeiss, Jena).

B b) c) Fungal cultur

The primary culture was carried out as a stab culture with Sabouraudglucose agar with chloramphenicol (Bio-Rad Laboratories GmbH, Munich) or Sabouraud-glucose agar with chloramphenicol and cycloheximide (Bio-Rad Laboratories GmbH, Munich). The cultures were incubated at room temperature for up to 4 weeks and monitored weekly.Growth was followed by cultural differentiation (see below). Negative findings were made after 4 weeks. For the differentiation of dermatophytes and molds followed by a new selective Sabouraud glucose agar plate inoculated with primary culture fungal material and incubated for at least one week (at slow growth for 2 weeks) at room temperature. Subsequently, native preparations of the fungal elements were evaluated microscopically. Optionally, further cultural differentiation was carried out with D.T.M. agar (dermatophyte test medium, Merck, Darmstadt) or urea test (BBL Prepared Culture Medium, Becton Dickinson GmbH, Heidelberg) according to the manufacturer

For the differentiation of yeasts, CandidaSelect ™ 4 (CE-IVD, Bio-Rad Laboratories GmbH, Munich), Reisagar (Merck, Darmstadt) and AuxaColor ™ 2 Yeast Identification System (CE-IVD, Bio-Rad Laboratories GmbH, Munich) were used according to the manufacturer used.

B b) d) DNA-extraction and purification

The DNA extraction was carried out with the QIAamp® DNA Mini Kit (Qiagen GmbH, Hilden) (see instructions for use) of the Mentype® **Mycoderm**^{QS} Lateral Flow PCR amplification kit).

B b) e) PCR

These experimental steps for multiplex PCR were performed as described in the instructions for use of the Mentype® **Mycoderm**^{QS} Lateral Flow PCR amplification kit.

B c) Results and discussion

240 patients with skin, mucosal and nail lesions were examined. Of these, 34 were already treated with an antimycotic drug. The clinical examination of the patients revealed 82 mycoses (tinea) and 48 onychomycoses. 272 findings were suspected without mycosis, of which 7 were already treated with an antimycotic.

According to the clinical findings, 137 nail chips, 175 dander and 90 direct swab smears were processed (sum 402), whereby patients with several different affected skin areas were sampled several times.

Of the 402 samples, 106 (26.4 %) were scored microscopically, 91 (22.6 %) culturally, 132 (32.8 %) microscopically and / or culturally, and 144 (35.8 %) in PCR positive. The consistency of the investigation methods is summarized in Table 1 below. The microscopically positive findings were confirmed to be 61.3 % by culture and 100 % by PCR. In positive cultures, 71.4% had positive microscopy, while 96.7 % could be confirmed by PCR.

The specific challenges of evaluating PCR tests for the diagnosis of dermatophytes have recently been reviewed in the literature [2]. In particular, the definition of a calibration standard is difficult because microscopy and culture are described only with sensitivities of 50-80 % and a large variance between different laboratories [3, 4]. Kondori et al. [5] have therefore suggested that all samples that are microscopically and / or culturally positive should be considered really positive. Table 2 summarizes the diagnostic values based on this definition of the reference standard.

Tab. 1: Consistency of the examination methods

Combined statement of diagnostic methods			Results	
microskopy	culture	PCR	number	%
-	-	-	255	63,4
+	+	+	65	16,2
+	+	-	0	0,0
-	+	+	23	5,7
+	-	+	41	10,2
-	+	-	3	0,7
+	-	-	0	0,0
-	-	+	15	3,7

Tab. 2: Diagnostic values for the PCR detection of dermatophytes in comparison to microscopy and / or culture as a reference method

		mikroskopy and/or culture				
		positive	negative	total		
PCR	positive	129	15	144	89,6	PPV [%]
	negative	3	255	258	98,8	NPV [%]
	total	132	270	402		
		97,7	94,4			
		DSE [%]	DSP [%]			

DSE, diagnostic sensitivity; DSP, diagnostic specifity;
PPV, positive predictive value, NPV, negative predictive value.

B d) References

[1] William A, Burke WA, Jones BE. A simple stain for rapid office diagnosis of fungus infections. *Archives of Dermatol 1984; 120: 1519-20.*

[2] Gräser Y, Czaika V, Ohst T. Diagnostic PCR of dermatophytes - an overview. *J Dtsch Dermatol Ges 2012; 10: 721-5.*

[3] Turin L, Riva F, Galbiati G, Cainelli T. Fast, simple and highly sensitive double-rounded polymerase chain reaction assay to detect medically relevant fungi in dermatological specimens. *Eur J Clin Invest 2000; 30: 511-8.*

[4] Summerbell RC, Cooper E, Bunn U, Jamieson F, Gupta AK. Onychomycosis: a critical study of techniques and criteria for confirming the etiologic significance of nondermatophytes. *Med Mycol 2005; 43: 39-59.*

[5] Kondori N, Abrahamsson AL, Ataollahy N, Wenneras C. Comparison of a new commercial test, Dermatophyte-PCR kit, with conventional methods for rapid detection and identification of *Trichophyton rubrum* in nail specimens. *Med Mycol 2010; 48: 1005-8.*

[6] Hoog GS, Dukik k, Monod M et al. New Nomenclatur.*Mykopathologia 2017; 182:5*

Appendix 1: Reference strains used and their reactivity in multiplex PCR 1, 2 and 3.
* CBS, **Centraalbureau voor Schimmelcultures**, Utrecht, NL. DSM, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig. ATCC, American Type Culture Collection über LGC Standards GmbH, Wesel. M, Dr. Mauersberger, Dresden.

Species	Trunk*	Multiplex PCR
Alternaria mali	CBS 106.24	-
Trichophyton benhamiae[1]	CBS 280.83	1 + 2
Arthroderma insingulare	CBS 521.71	1
Arthroderma lenticulare	CBS 307.65	1
Arthroderma quadrifidum	CBS 613.74	1
Arthroderma vanbreuseghemii	CBS 646.73	1 + 2
Aspergillus flavus	N 203979/2008	-
Aspergillus flavus	M S06	-
Aspergillus fumigatus	N 120293/2006	-
Aspergillus fumigatus	M S07	-
Aspergillus niger	N 200939/2009	-
Aspergillus niger	M S05	-
Aspergillus niger	N 201250/2009	-
Aspergillus versicolor	CBS 583.65	-
Aspergillus versicolor	DSM 1943	-
Aspergillus versicolor	M S12	-
Bacillus subtilis	DSM 10	-
Candida albicans	N 08/2008 Strain C	1
Candida albicans	N 107441/2009	1
Candida albicans	DSM 1386	1
Candida glabrata	ATCC 90030	1
Candida glabrata	N 2005-5	1
Candida glabrata	DSM 6425	1
Candida guilliermondii	N 490-1/2009-2	1
Candida guilliermondii	DSM 6381	1
Candida krusei	ATCC 6258	1
Candida krusei	N 490-1/2007-3	1
Candida krusei	DSM 3433	1
Candida parapsilosis	N 107206/2009	1
Candida parapsilosis	N 107577/2009	1
Candida parapsilosis	DSM 5784	1
Candida tropicalis	N 102744/2008	1
Candida tropicalis	N 2/2008-3	1
Candida tropicalis	DSM 11953	1
Enterococcus faecalis	DSM 24916	-

Species	Trunk*	Multiplex PCR
Alternaria mali	CBS 106.24	-
Epidermophyton floccosum	N 114327/2007	1 + 3
Epidermophyton floccosum	N 204179/2008	1 + 3
Epidermophyton floccosum	CBS 358.93	1 + 3
Escherichia coli	ATCC 25922	-
Fusarium oxysporum	DSM 2018	-
Fusarium solani	DSM 62416	-
Malassezia furfur	CBS 1878	-
Malassezia globosa	CBS 7966	-
Malassezia restricta	CBS 7877	-
Microsporum canis	CBS 282.63	1 + 3
Microsporum canis	N 203213/2007	1 + 3
Microsporum canis	CBS 190.57	1 + 3
Nannizzia gypsea[2]	N 203237/2007	1 + 3
Nannizzia gypsea[2]	N 203353/2002	1 + 3
Nannizzia gypsea[2]	CBS 258.61	1 + 3
Microsporum audouinii	CBS 280.63	1 + 3
Microsporum audouinii	CBS 344.50	1 + 3
Microsporum audouinii	CBS 119449	1 + 3
Microsporum ferrugineum	CBS 457.80	1
Microsporum ferrugineum	CBS 426.63	1
Penicillium chrysogenum	DSM 848	-
Penicillium griseovulvum	DSM 896	-
Rhodotorula glutinis	DSM 70398	-
Rhodotorula mucilaginosa	DSM 70404	-
Saccharomyces cerevisiae	DSM 70449	1
Scopulariopsis brevicaulis	N 11/06	1
Scopulariopsis brevicaulis	N 2002-C	1
Scopulariopsis brevicaulis	DSM 9122	1
Staphylococcus aureus	DSM 20231	-
Staphylococcus epidermidis	DSM 20044	-
Trichophyton interdigitale	N 200454/2009	1 + 2
Trichophyton interdigitale	N 201029/2008	1 + 2
Trichophyton interdigitale	CBS 558.66	1 + 2
Trichophyton mentagrophytes	CBS 106.67	1 + 2
Trichophyton rubrum	N 107241/2009	1
Trichophyton rubrum	N 107246/2009	1
Trichophyton rubrum	CBS 392.58	1
Trichophyton tonsurans	CBS 120.65	1 + 2
Trichophyton tonsurans	CBS 483.76	1 + 2

Species	Trunk*	Multiplex PCR
Alternaria mali	CBS 106.24	-
Trichophyton tonsurans	CBS 496.48	1 + 2
Trichophyton verrucosum	CBS 134.66	1 + 2
Trichophyton verrucosum	CBS 554.84	1 + 2
Trichophyton verrucosum	CBS 564.50	1 + 2
Trichophyton violaceum	CBS 319.31	1 + 2
Trichophyton violaceum	CBS 730.88	1 + 2
Trichosporon cutaneum	DSM 70675	-
Trichosporon cutaneum	DSM 70698	-

[1-2] Formerly: [1]T. Species von *A. benhamiae* [2]*M. gypseum*. [6]