Dermatophyte PCR Kit

PCR Kit for detection of dermatophytes and *Trichophyton rubrum*

For *in vitro* diagnostic use only

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Application

The Dermatophyte Nail PCR is to be used for in vitro diagnostic detection of dermatophytes in general (pan-dermatophytes) and specifically *Trichophyton rubrum*.

Description

The Kit includes two buffers for DNA preparation, PCR ReadyMix (including loading buffer), primer mix and two control DNA samples. The primer mix contains two primer pairs directed towards genes encoding chitin synthase 1 for detection of dermatophytes in general and ITS2 (internal transcribed spacer) for detection of *T. rubrum*. All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'- hydroxyl ends. The individual concentration of each primer pair is adjusted for optimal performance in the multiplex PCR. An internal plasmid control that serves as template for the *T. rubrum* specific primers is added to the primer mix. Control 1 consists of dermatophyte genomic DNA and control 2 consists of *T. rubrum* genomic DNA. The package contains reagents enough to perform 100 multiplex PCR reactions.

Background

Nail infections are mainly caused by *T. rubrum*, followed by *T. mentagrophytes*. Traditionally the time required for species identification by culture may vary from 10 to 15 days up to 3 to 4 weeks[1]. This PCR based diagnostic method can detect dermatophytes in general and specifically *T. rubrum* within 5 hours.

The amplicon sizes for identification of a dermathophyte in general or a *T. rubrum* are listed below.

Gene	Detects	Amplicon size (bp)
chs1	Pan-dermatophytes	366
its2	*T. rubrum*	203
T. rubrum DNA	Internal plasmid control	700

Materials required but not provided

2.0% agarose gel
DNA marker

Procedure

Sample preparation and PCR setup should be performed in dedicated areas free of possible contamination.

DNA preparation

1. The nail specimen is incubated at 95ºC for 10 min in 100 µL Buffer A*.
2. Immediately add 100 µL Buffer B and vortex. The sample is ready for PCR.

* If the nail sample is large the volume of buffer A should be increased to cover the sample. The volume of Buffer B is increased equally.

PCR setup

3. Prepare the total master mix (PCR ReadyMix and primer mix) for the number of samples to be run and dispense 16 µL of the mixture in each tube. Each tube has to contain the following amount of reagents:

	Nail sample	Positive *T. rubrum* control	Positive Pan-derm control	Negative control
PCR ReadyMix*1	10.0 µL	10.0 µL	10.0 µL	10.0 µL
Primer mix	6.0 µL	6.0 µL	6.0 µL	6.0 µL
Nail sample	4.0 µL*2	-	-	-
T. rubrum DNA	-	4.0 µL	-	-
Pan-derm DNA	-	-	4.0 µL	-
Buffer-mix*3	-	-	-	4.0 µL
Total	20.0 µL	20.0 µL	20.0 µL	20.0 µL

*1Procuct from Sigma-Aldrich
*2Occasionally the nail sample suppress the DNA amplification in the PCR reaction. This is observed as no bands on the agarose gel. In such case repeat step 3 as described but reduce the volume of nail sample to 2 µL.
*3Mix buffer A and buffer B in the ratio 1:1.

4. Run the PCR amplification in a thermocycler at the following conditions.

Step	Temp. (ºC)	Time
Initial denaturation	94	5 min
45 cycles of:		
Denaturation	94	30 sec
Annealing	60	30 sec
Extension	72	30 sec
Final extension	72	3 min

5. Run 15 µL of each completed PCR in separate wells on a 2% agarose gel capable of separating the particular amplicon sizes.

Interpretation of the analysis results

The figure below shows the PCR results compared to a 100 bp DNA ladder. A *T. rubrum* positive nail specimen will often give a strong band of 203 bp and a weaker or no band at 366 bp due to the relative higher copy number of *its2* compared to *chs1*. Likewise a positive nail specimen often results in no or a weak internal control band due to relative high concentrations of pan-dermatopytes.

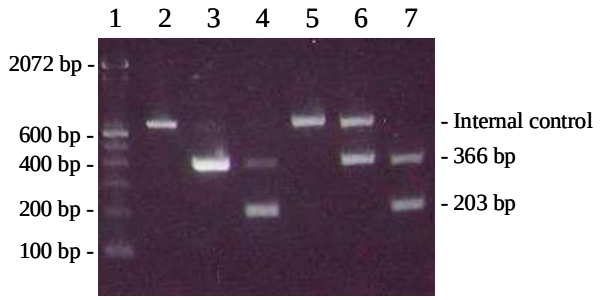

Figure 1. *T. rubrum* specific and pan-dermatophyte multiplex PCR product analysis. Lane 1: Molecular size marker (100 bp DNA ladder); Lane 2: Negative control; Lane 3: Dermatophyte genomic DNA; Lane 4: *T. rubrum* genomic DNA; Lane 5: Negative nail specimen; Lane 6: Pan-dermatophyte positive nail specimen; Lane 7: *T. rubrum* and pan-dermatophyte positive nail specimen.

Specificity

A total of 118 nail specimens were tested for pan-dermatophyte and *T. rubrum* infection by both the multiplex PCR method and the conventional methods (microscopy and/or culture). Overall, 42.4% of the specimens were pan-dermatophyte positive by PCR whereas 38.1% were positive using the conventional methods. The sensitivity of pan-dermatophyte identification in nail specimens was therefore increased by 4.3% using the multiplex PCR method. Furthermore, the test showed that the sensitivity for identification of *T. rubrum* was increased by 18.6% using the PCR based diagnostic (see table)[1].

	Pan-dermatophytes	*T. rubrum*
Conventional methods	38.1 %	22.9 %
PCR	42.4 %	41.5 %
Increased detection	**4.3 %**	**18.6 %**

Storage and shelf life

The PCR Kit should be stored at ÷20 ºC. Dispense the PCR ReadyMix, the primer mix and the DNA controls into several aliquots and store them at ÷20°C. Primer mix and control aliquots in use can be stored at 4-8 °C for up to two weeks.

The expiry date of the kit is printed on the label.

References

1. Brillowska-Dabrowska, A., Saunte, D. M. and Arendrup, M. C. 2007. Five-Hour Diagnosis of Dermatophyte Nail Infection with Specific Detection of *Trichophyton rubrum*. Jour. Clin. Microbiol. 1200-1204.

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