EurobioPlex

Dermatophytes

REAL-TIME PCR

For **qualitative** real-time PCR

REF **EBX-023**

48/96/192 reactions

CE IVD

Validated on:

- CFX96™ Real Time PCR detection system (Biorad) with analysis on CFX Manager v 3.1 (Biorad)
- LightCycler®480 Instrument II (Roche) with analysis on LightCycler® 480 software v1.5 (Roche)

V1.00

Storage conditions:

Keep all reagents between -15°C and -22°C until use and after first use

Instructions for use

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INTENDED USE

The Dermatophytes test uses real-time polymerase chain reaction (PCR) amplification, and is designed for the qualitative detection of the six most frequently involved dermatophytes in superficial cutaneous mycosis of the skin, hair and nails (dermatophyties): *Trichophyton rubrum/violaceum* (undifferentiated detection of these two species), *Trichophyton tonsurans, Trichophyton mentagrophyte var. interdigitale*, *Microsporum canis,* and *Epidermophyton floccosum*, from a DNA extract. This test is indicated to confirm a diagnosis of presumption of infection in patients or complete a proven or indeterminate diagnosis by other techniques such as direct examination and culture (identification according to the time of growth, macroscopic and microscopic examinations). Extracted DNA is the starting material for the Eurobioplex Dermatophytes kit.

The Eurobioplex Dermatophytes has been validated on the following specimen:

- Pure cultures of dermatophytes fungi isolated from clinical samples
- Mycological clinical samples (swabs rubbed on the scalp, nails, skin, hair and dander)

INTRODUCTION

Dermatophytes are filamentous fungi belonging to the genera *Microsporum* (M.), *Trichophyton* (T.), and *Epidermophyton* (E.). They have an affinity for the keratin of skin, hair, and nails producing various clinical forms: lesions of the skin or epidermophyties, tinea capitis for injury of the scalp, folliculitis for the invasion of the hair by a dermatophyte, onychomycosis for nail affection. The onychomycosis are disorders frequently encountered in dermatology and constitute up to 50% of the onychopathies. Their prevalence is increasing in recent years.

Many pathogens can be the cause of these dermatophyties. They are characterized by various spore productions, and are divided into 3 groups:
1 / anthropophiles, exclusive human parasites, transmitted by direct contact, clothes, ground. *T. rubrum* is the most common (found in 70-90% of the dermatophytosis), followed by *T. mentagrophyte interdigitale* and *T. tonsurans* that belong to the T. mentagrophyte complex. *T. violaceum*, which belongs to the *T rubrum complex*, and *E. floccosum* are also part of anthropophiles species.
2 / anthropo-zoophiles, that are transmitted to humans by a contaminated animal (claws, hair; ex: *M. canis, M. equinum, M. gallinae, M. persicolor, T. equinum, T mentagrophyte var mentagrophyte, T. sarkisovii, T. simmii* and *T. verrucosum*). *M. canis*, transmitted by cats, is the most common of this group and is the second most frequent pathogen.
3 / geophiles or telluric found on the ground (ex: T. terrestre, M gypseum).

The frequency of each species varies depending on the country. In Northern Europe and North America, the main pathogens are *M. canis* and *T. rubrum*. Zoophiles dermatophytes are more common in Southern Europe and in Arabic countries. The main species found in metropolitan France are *T. rubrum* and *T. mentagrophyte var. interdigitale*.

There is no precise diagnosis for the identification of the dermatophyte responsible for the affection of keratin by the clinician. Diagnosis often does not allow differentiating species due to the morphological similarities of some of these dermatophytes. In case of suspected mycosis, a mycological sample with direct examination and culture is systematically done and even mandatory and ideally prior to any treatment. The treatment must be local in all cases and general and extended in cases of affection of the hair and nails. It is generally initiated without waiting for the results of the macro- tests (color and texture) and the post culture microscopic analysis (aspect of the filaments and spores) that last 3 to 5 weeks. Dermatophytes growth on Sabouraud media form filaments (mycorrhizal) and spores from an asexual reproduction called conidia. Depending on their genera, these fungi can produce microconidia (*M. spp, T. spp*) or not (*E. floccosum*). Therefore, an accurate diagnosis would permit to avoid additional costs and time lost because the treatment may be inefficient following a false diagnosis. The knowledge of the species allows specifying the origin of the contamination.

The test of gene amplification by polymerase chain reaction (PCR) is the fastest and most specific technique for the targeted diagnostics of dermatophytes. Although dermatophytes DNA present a strong homology of sequence, the multiplex EBX-023 allows amplifying specific DNA of the following dermatophytes: *T. rubrum/violaceum* (undifferentiated detection), *T. tonsurans, M. canis, T. mentagrophyte interdigitale*, and *E. floccosum*.

PRINCIPLE OF DETECTION

The Eurobioplex Dermatophytes is a test using real-time amplification of fungal DNA of *T. rubrum/violaceum* (undifferentiated detection of these two species), *T. tonsurans, M. canis, T. mentagrophyte interdigitale*, and *E. floccosum*, as well as a DNA extraction and PCR inhibition control. The test is performed from the DNA extracted from a sample using two reactions in two wells/tubes.

The DNA extraction and PCR inhibition control allows to check for variations that may occur during the DNA extraction step of biological samples and real-time PCR amplification. Thus, it ensures that a negative result may not be due to a bad DNA extraction and/or due to the presence of PCR inhibitors in too large quantities.

This kit contains two different oligomixes which permit to detect the different pathogens listed below:
Oligomix 1: *M. canis* (MC), *T. mentagrophyte interdigitale* (TI) + control of DNA extraction and PCR inhibition (CI-PCR);
Oligomix 2: *T. rubrum/violaceum* (TR + TV; undifferentiated detection), *T. tonsurans* (TT), and *E. floccosum* (EF) + control of DNA extraction and PCR inhibition (CI-PCR).
DNA of MC and TI is respectively detected using FAM and HEX labeled probes. DNA of (TR + TV) is detected using a FAM labeled probe, and DNA of TT and EF is respectively detected using HEX and Texas Red labeled probes. For the two multiplexes, DNA extraction and PCR inhibition control is detected using a CY5 labeled probe. All probes emit a specific fluorescence following their hydrolysis during the elongation of the amplification product. The measurement of the intensities of real-time fluorescence correlates with the accumulation of specific amplification products.

DESCRIPTION AND CONTENT OF THE KIT

The real-time PCR Dermatophyte kit is ready to use for the specific detection of *T. rubrum/violaceum* (undifferentiated detection), *T. tonsurans*, *M. canis, T. interdigitale,* and *E. floccosum*.

Fluorescence is emitted and individually recorded through optical measurements during the PCR. The detection of the amplified fragment is performed by a fluorimeter using the channels shown in Table 1 below.

The kit contains reagents and enzyme for the amplification of DNA of these fungi, and the DNA extraction and PCR inhibition control (see Table 2)

3 sizes are available for this kit:
- 48 reactions to test 22 patients samples with the two oligomixes
- 96 reactions to test 46 patients samples with the two oligomixes
- 192 reactions to test 94 patients samples with the two oligomixes.

Table 1 : Pathogens detected by each oligomix

Target	Oligomix 1	Oligomix 2	Fluorophore
Microsporum canis	X	-	FAM
Trichophyton interdigitale	X	-	HEX
Trichophyton rubrum + Trichophyton violaceum	-	X	FAM
Trichophyton tonsurans	-	X	HEX
Epidermophyton floccosum	-	X	Texas Red
PCR inhibition control (CI-PCR)	X	X	Cy5

Wavelengths of excitation/emission for FAM (495/515 nm), HEX (535/555 nm), Texas Red (585/605 nm), CY5 (647/667 nm).

Equivalent channels on different real time PCR cyclers:
- Channel **FAM** (ABI Systems, SmartCycler II, Chromo 4/CFX96, Systems Mx), Channel 510 (LC 480), Channel Green (RotorGene)
- Channel **HEX** (Chromo 4/CFX96, Mx Systems), Channel VIC (ABI Systems), Channel Alexa532 (SmartCycler II), Channel 580 (LC 480), Channel Yellow (RotorGene),
- Channel **Texas Red**: LC Red 610 (LC480), Channel Orange (RotorGene)
- Channel **Cy5** (ABI Systems, Chromo 4/CFX96, Mx Systems), Channel 660 (LC 480), Channel Alexa647 (SmartCyclerII), Channel Red (RotorGene)

Note: On LC480 instrument II, apply color compensation for the following channels: FAM-HEX/VIC-TexasRed-Cy5 (465-510, 533-580, 533-610, 618-660).

Table 2:

Cap color	Components of the kit	192 reactions	96 reactions	48 reactions	Reconstitution
Red	Enzyme	8 x 375 µl	4 x 375 µl	2 x 375 µl	Ready to use
Transparent	Oligomix 1	4 x 210 µl	2 x 210 µl	210 µl	Ready to use
Green	Oligomix 2	4 x 210 µl	2 x 210 µl	210 µl	Ready to use
Blue	Water = negative control (CN-H2O)	1 ml	1 ml	1 ml	Ready to use
Yellow	Positive Control Dermatophytes 1 (CP1)	4 x 40 µl	2 x 40 µl	40 µl	Ready to use
Orange	Positive Control Dermatophytes 2 (CP2)	4 x 40 µl	2 x 40 µl	40 µl	Ready to use
White	PCR inhibition control (CI-PCR)	4 x 300 µl	2 x 300 µl	300 µl	Ready to use

Oligomix1: contains the primers and probes of the triplex MC+TI+CI-PCR
Oligomix2: contains the primers and probes of the quadriplex (TR+TV)+TT+EF+CI-PCR

Required material not provided:

- ◊ Biological Hood
- ◊ Real-time PCR instrument
- ◊ Micro centrifuge
- ◊ Vortex
- ◊ Plates / tubes for real-time PCR
- ◊ Micropipettes
- ◊ DNase-free RNase-free filter tips for micropipettes
- ◊ Sterile microtubes
- ◊ Gloves (powder free)

STORAGE

All reagents must be stored between -15 and -22°C.
All reagents can be used until the expiration date indicated on the kit.
Many freezing / defrosting cycles (> 3x) must be avoided, and could lead to decrease in sensitivity.

CAUTIONS AND NOTES

Read carefully instructions before starting.

- ◊ The experiment must be performed by competent staff.
- ◊ Instruments must have been properly installed, calibrated and maintained according to the manufacturer's recommendations.
- ◊ Clinical samples are potentially infectious and must be processed under a laminar flow hood.
- ◊ The experiment must be performed according to good laboratory practices.
- ◊ Do not use this kit after expiration date.
- ◊ The kit is shipped with dry ice, and the components of the kits must arrive frozen. If one or more components are defrosted, or of the tubes have been damaged, contact Eurobio-Ingen.
- ◊ Once defrosted, spin down briefly the tubes before use.
- ◊ Use of ice or cooling block is advised in case of long delay du for instance to large number of samples or high temperature.
- ◊ It is recommended to define three working areas: 1) Isolation of DNA, 2) Preparation of the reaction mix and 3) Amplification / Detection of amplified products.
- ◊ Use specific lab coat and gloves (powder free) in each working area.
- ◊ Pipettes, reagents and other materials must not cross each area.
- ◊ Specific caution is required to preserve the purity of the reagents and reaction mixtures.
- ◊ Appropriate methods of preparation/extraction of DNA to produce high quality DNA and to be followed by a real-time PCR application should be used, particularly avoiding all sources of DNase contamination.
- ◊ Always use RNase-free DNase-free filtered tips for micropipettes.
- ◊ Do not pipette with mouth and do not eat, drink or smoke in the area.
- ◊ Avoid sprays.

SAMPLES COLLECTION, TRANSPORT AND STORAGE

- ◊ Collect samples in sterile containers (for example: Petri dish).
- ◊ It is the responsibility of the user to master its own conditions of collection, transport and storage of samples, and extraction of DNA by suitable systems to produce DNA of good quality.
- ◊ It is recommended that samples be stored according to the recommendations of storage of samples before extraction (Table 3).

Table 3 :

Recommendations of maximum storage of samples before extraction	
Room temperature	1 month

- ◊ The user can refer to the World Health Organization or High Health Authority for storing samples.
- ◊ Transport of clinical samples must follow local regulations for this type of infectious agents.

PROCEDURE

I- DNA extraction

It is the user's responsibility that the extraction system used be compatible with downstream types of specimen and real time PCR technology. For this kit, you can use your own extraction technique or a suitable commercial system by referring to the manufacturer's instructions. Generally commercial kits for skin or nail samples use a pre-treatment consisting of an incubation under agitation of the sample in the presence of lysis buffer, of proteinase K, and/or silica beads, sometimes followed by an homogenization. The DNA must then be extracted.

The kit is intended for use on extracted DNA, and it is the user responsibility to validate his extraction method of choice.

In the Dermatophytes kit, CI-PCR on the CY5 channel can be added before extraction or in the PCR reaction. It ensures that a negative result is not due to an extraction problem or due to the presence of PCR inhibitors at high quantity.
We recommend the addition of 10 µl of CI-PCR per extraction. Then, add 5µl/reaction of PCR from a final volume of elution of 50µl after extraction. If the CI-PCR is added to control the real-time PCR, CI-PCR is added to the reaction mix (1 µl per PCR reaction). See real-time PCR protocol for details.

CI-PCR is also available separately from Eurobio (Ref EurobioPlex EBX-002).

II- Real-time PCR procedure

General comment:

The Dermatophytes Positive Controls CP1 and CP2, and the DNA extraction and PCR inhibition control (CI-PCR) contain a very high concentration of matrix. Manipulations must be performed carefully to avoid contamination. To control the functioning of the PCR and steps of extraction and real-time PCR amplification, it is necessary to test the CP1 and CP2 positive controls with the corresponding oligomix, as well as a negative control (water supplied = CN-H2O + CI-PCR) (see II-2/6) of real-time PCR procedure).

II-1/ Diagram of the procedure

1 – PREPARATION OF REACTION MIXES / MASTERMIXES

(a) For a DNA sample extracted WITHOUT CI-PCR

Number of reaction(s)	N+3
Enzyme	(N+3) x 12.5 µl
Oligomix 1 or 2	(N+3) x 7.5 µl
CI-PCR	(N+3) x 1 µl
Total Volume Mastermix 1 or 2	(N+3) x 21 µl

(b) For a DNA sample extracted WITH CI-PCR

Number of reaction(s)	N+3
Enzyme	(N+3) x 12.5 µl
Oligomix 1 or 2	(N+3) x 7.5 µl
Total Volume Mastermix 1 or 2	(N+3) x 20 µl

2 - PREPARATION OF REACTIONS

Sample

20 µl Mastermix 1 or 2
+
5µl DNA sample

Positive Controls

20 µl Mastermix 1
+
5µl CP1

20 µl Mastermix 2
+
5µl CP2

Negative Controls

20 µl Mastermix 1 or 2
+
5µl supplied water (CN-H2O)

Sample

20 µl Mastermix 1 or 2
+
5µl DNA sample

Positive Controls

20 µl Mastermix 1
+
5µl CP1

20 µl Mastermix 2
+
5µl CP2

Negative Controls

20 µl Mastermix 1 or 2
+
4µl supplied water (CN-H2O)
+
1 µl CI-PCR

3 – REAL-TIME PCR INSTRUMENT

Program	Temperature	Duration	Cycle(s)	
Denaturation	95°C	3 min	1	-
Amplification	95°C	10 sec	40	-
	56°C	30 sec		-
	72°C	45 sec		Fluorescence Acquisition

II-2/ Detailed Procedure

1) Homogenize the tube of Enzyme, and vortex Oligomix 1, Oligomix 2, CP1, CP2, and CI-PCR tubes before starting, and centrifuge.
2) Prepare Mastermixes 1 and 2 as below; N is the number of PCR reactions. Plan to prepare enough reagents for at least N+3 reactions (refer to part 1-(a) or 1-(b) of the previous diagram according to the case).

Case (a): for a DNA sample extracted without CI-PCR

Number of reaction(s)	**1**	**N+3**
Enzyme	12.5 µl	(N+3) x 12.5 µl
Oligomix 1 or 2	7.5 µl	(N+3) x 7.5 µl
CI-PCR	1 µl	(N+3) x 1 µl
Total Volume Mastermix 1 or 2	21 µl*	(N+3) x 21 µl

Case (b): for a DNA sample extracted WITH CI-PCR

Number of reaction(s)	**1**	**N+3**
Enzyme	12.5 µl	(N+3) x 12.5 µl
Oligomix 1 or 2	7.5 µl	(N+3) x 7.5 µl
Total Volume Mastermix 1 or 2	20 µl*	(N+3) x 20 µl

** The volume difference between case (a) or (b) has no effect on performance.*

3) Homogenize the mastermixes prepared in 2) and centrifuge briefly
4) Distribute 20 µL Mastermix* 1 or 2 using a micropipette and filtered tips in each tube or well of microplate for real time PCR.
5) Add 5 µL of extracted DNA sample.
6) In parallel, test the following controls:
   - Positive controls:
     - 20µL of mastermix 1 + 5µL of CP1
     - 20µL of mastermix 2 + 5µL of CP2
   - Negative controls:
     - Case (a): for a DNA sample extracted without CI-PCR
       - 20 µL mastermix 1 + 5 µL supplied water (CN-H2O)
       - 20 µL mastermix 2 + 5 µL supplied water (CN-H2O)
     - Case (b): for a DNA sample extracted with CI-PCR
       - 20 µL mastermix 1 + 4 µL water supplied (CN-H2O) + 1 µL CI-PCR
       - 20 µL mastermix 2 + 4 µL water supplied (CN-H2O)+ 1 µL CI-PCR
7) Close immediately with an adhesive film or transparent caps to avoid contamination.
8) Centrifuge briefly to collect all the PCR reaction mix at the bottom of the tubes or plate.
9) Program the real-time PCR instrument as follows.

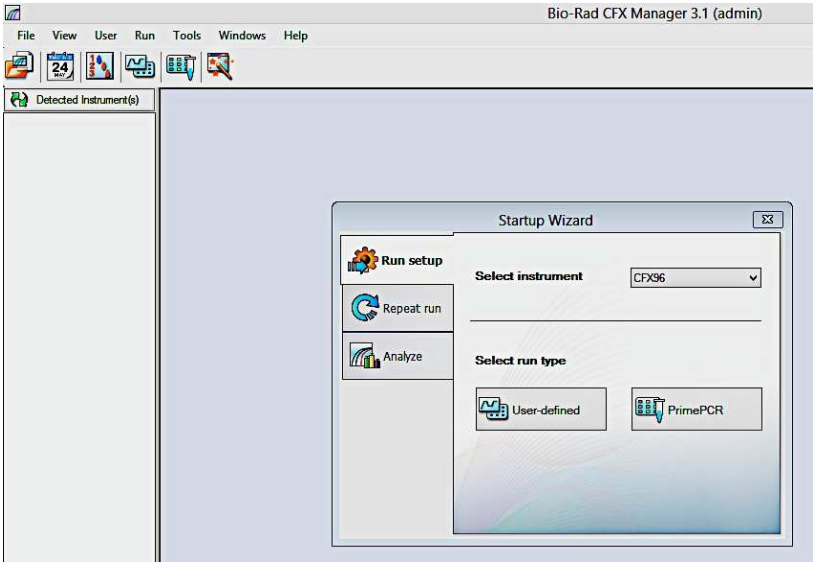
Program	Temperature	Duration	Cycle(s)	
Denaturation	95°C	3 min	1	-
Amplification	95°C	10 sec	40	-
	56°C	30 sec		-
	72°C	45 sec		Fluorescence Acquisition

Note 1: On LightCycler ® 480 systems (Roche), two optical systems are available: only "System II" is compliant with use of the kit. Apply color compensation for the following channels: FAM-HEX/VIC-TexasRed-Cy5 (465-510, 533-580, 533-610, 618-660).
Note 2: For the Applied Biosystems systems, select “NONE” in "PASSIVE REFERENCE".
Note 3: On Rotorgene ™, please calibrate the signal by clicking on “GAIN optimization”.
Note 4: On CFX96 (Biorad), start the run using the v1.6 or later version of CFX Manager software, and analyse with v 3.1 (see § Validation of the Experiment)

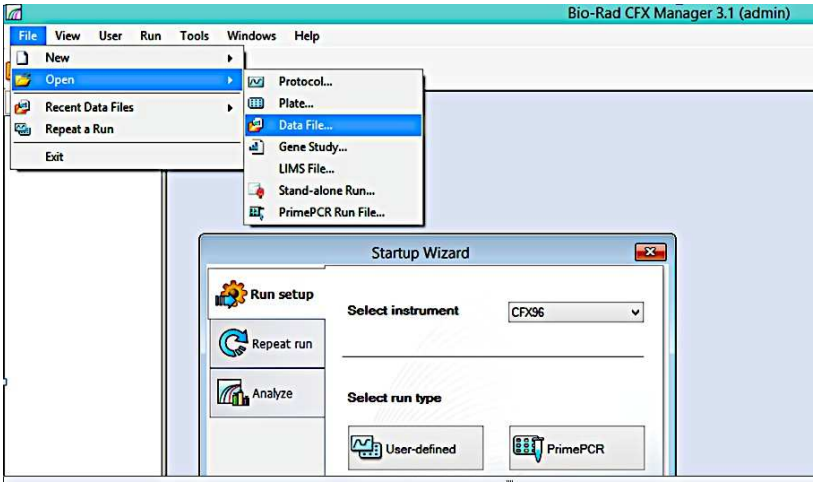
VALIDATION OF THE EXPERIMENT

The analysis of data post-acquisition on a CFX96 PCR instrument (Biorad) must be done with version 3.1 of CFX Manager Software (Biorad). In order to use this v3.1 version from a run started with an older version, follow the procedure below: at the end of the run, the data file with .pcrd suffix must be open and treated with version 3.1 of CFX Manager (Biorad).

If the run was done with CFX Manager v1.6 for instance, to open the data file with CFX Manager v3.1, click on CFX Manager v3.1 icon. The screen below appears.

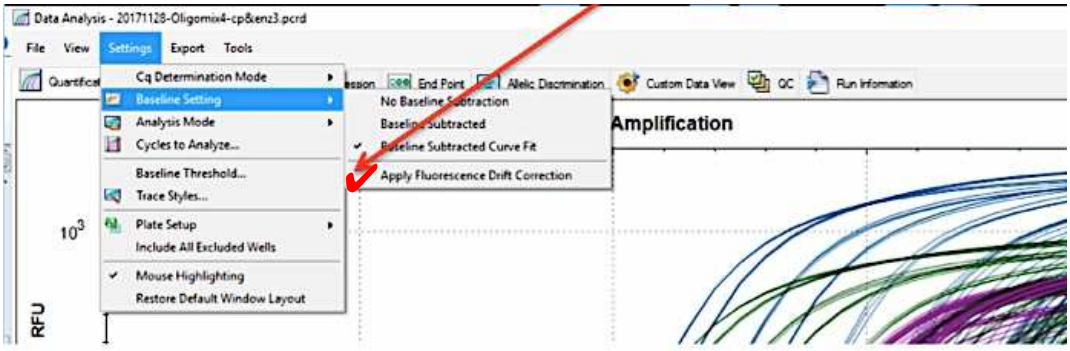


- Click on File and select Open, then Data File



- Select the file you want to analyze and click on Open.

The « drift correction » option must be selected from the « Settings » Tab, as indicated on the image below: click on « Settings », then « Baseline Setting » then on « Apply Fluorescence Drift Correction ».



Once this is done, the analysis can start.

For the assay to be valid, the results for the controls must be the following (Table 4). Otherwise, the experiment is not valid.

Table 4:

Positive Controls		
Channel	CP1	CP2
FAM	POSITIVE (Ct ≤ 40)	POSITIVE (Ct ≤ 40)
HEX	POSITIVE (Ct ≤ 40)	POSITIVE (Ct ≤ 40)
TEXAS RED	Not applicable	POSITIVE (Ct ≤ 40)
Negative Controls		
Channel	Negative Control Mastermix 1	Negative Control Mastermix 2
FAM	Undetermined	Undetermined
HEX	Undetermined	Undetermined
TEXAS RED	Not applicable	Ct ≥ 37 or undetermined
CY5	POSITIVE (Ct ≤ 40)	POSITIVE (Ct ≤ 40)

CP1: Dermatophytes Positive Control for Multiplex 1
CP2: Dermatophytes Positive Control for Multiplex 2

DATA ANALYSIS AND INTERPRETATION

DNA extraction and PCR inhibition control in biological samples:

Two results can be obtained:

1/ the DNA extraction and PCR inhibition control (CI-PCR; channel Cy5) test is positive: DNA has been properly extracted, and there is no PCR inhibitor. The result can be validated.

2/ the DNA extraction and PCR inhibition control (CI-PCR; channel Cy5) test is negative: either DNA was not extracted, the PCR did not work well, or the presence of PCR inhibitors inhibits the PCR reaction. It is then recommended to repeat the extraction or dilute the sample, unless a specific signal appears in the targeted channels (FAM, HEX, Texas Red).

OLIGOMIX 1:

For clinical samples tested with the Oligomix 1, the following results are possible:

*** Ct cut off for positivity of samples:**

Channel FAM *Microsporum canis* **: + Positive => Positive Ct (≤ 40)**

Channel HEX *Trichophyton interdigitale* **: + Positive => Positive Ct (≤ 40)**

PCR Signal			Presence of MC	Presence of TI	Test validity/comment
FAM	HEX	CY5			
+*	+*	+	Yes	Yes	**valid**
+*	-	+	Yes	No	**valid**
-	+*	+	No	Yes	**valid**
-	-	+	No	No	**valid**
+*	+*	-	Yes	Yes	**Valid**: Possible inhibition of PCR or problem with extraction that does not prevent the detection of MC and TI.
+*	-	-	Yes	No possible interpretation	**Valid for MC**: Possible inhibition of PCR or problem with extraction that does not prevent the detection of MC **Not valid for TI**: Possible inhibition of PCR or problem with extraction that would prevent the detection of TI → dilute 5 x the sample. If necessary redo an extraction.
-	+*	-	No possible interpretation	Yes	**Valid for TI**: Possible inhibition of PCR or problem with extraction that does not prevent the detection of TI **Not valid for MC**: Possible inhibition of PCR or problem with extraction that would prevent the detection of MC → dilute 5 x the sample. If necessary redo an extraction.
-	-	-	No possible interpretation	No possible interpretation	**Not valid:** Inhibition of PCR or problem with extraction - dilute first 5 x the sample ; if necessary redo an extraction

MC : *Microsporum canis;* TI : *Trichophyton interdigitale*

OLIGOMIX 2:

For clinical samples tested with the Oligomix 2, the following results are possible:

*** Ct cut off for positivity of samples:**
channel FAM-TR+TV: + Positive => Positive Ct (≤ 40)
channel HEX-TT: + Positive => Positive Ct (≤ 40)
channel Texas Red-EF: + Positive => Positive Ct and < 37

PCR Signal				Presence of TR or TV	Presence of TT	Presence of EF	Test validity/comment
FAM	HEX	Texas Red	CY5				
+*	+*	+*	+	Yes	Yes	Yes	**VALID**
+*	+*	-	+	Yes	Yes	No	
+*	-	+*	+	Yes	No	Yes	
-	+*	+*	+	No	Yes	Yes	
+*	-	-	+	Yes	No	No	
-	+*	-	+	No	Yes	No	
-	-	+*	+	No	No	Yes	
-	-	-	+	No	No	No	
+*	+*	+*	-	Yes	Yes	Yes	**Valid**: Possible inhibition of PCR or problem with extraction that does not prevent the detection of TR or TV, TT and EF
+*	-	-	-	Yes	No possible interpretation	No possible interpretation	Possible PCR inhibition that does not prevent the detection of one or several pathogens (noticed by "Yes") ; valid for this pathogen(s) BUT possible PCR inhibition that would prevent the detection of other targeted pathogen(s) → dilute 5 x the sample; if necessary redo an extraction.
-	+*	-	-	No possible interpretation	Yes	No possible interpretation	
-	-	+*	-	No possible interpretation	No possible interpretation	Yes	
+*	+*	-	-	Yes	Yes	No possible interpretation	
+*	-	+*	-	Yes	No possible interpretation	Yes	
-	+*	+*	-	No possible interpretation	Yes	Yes	
-	-	-	-	No possible interpretation	No possible interpretation	No possible interpretation	**Not valid:** Possible inhibition of PCR or problem with extraction - dilute first 5 x the sample ; if necessary redo an extraction

TR+TV : *Trichophyton rubrum + Trichophyton violaceum* (cross detection for the two species)
TT : *Trichophyton tonsurans*
EF : *Epidermophyton floccosum*

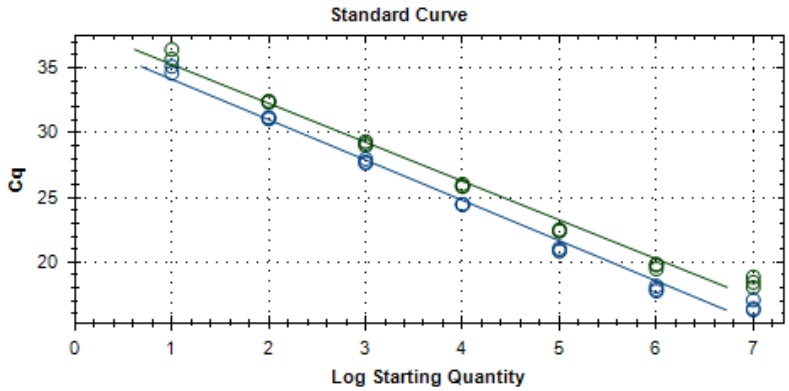
Note that because of the strong nucleic homology observed between dermatophytes, positive results do not exclude the possibility of co-infection by other dermatophytes species which have not been specifically targeted at the development of this kit.

For example, it is possible that:
- some strains of the *Arthoderma otae* complex such as *M. ferrugineum* and *M. equinum*, may be recognized by the system of detection of *M. canis*,
- *T. soudanense, T. gourvilii, T. circonvolutum* may be recognized by the design of *T. rubrum*. *T. rubrum* presents a cross-detection demonstrated with *T. violaceum*,
- *T. equinum* may be recognized by *T. tonsurans*,
- *Arthroderma benhamiae, Arthroderma vanbreuseqhemii* may be recognized by the design of *T. mentagrophyte interdigitale*.

PERFORMANCE ANALYSIS

Example of experiment performed on real-time PCR thermocycleur CFX96 (Biorad):

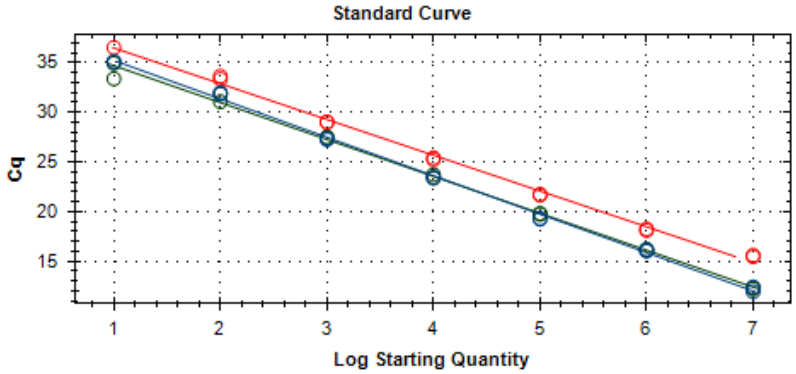
With Oligomix 1:



- FAM : *M. canis*

- HEX : *T. interdigitale*

With Oligomix 2:



- **FAM : *T. rubrum + T. violaceum* (not differenciated)**
- **HEX : *T. tonsurans***
- **TEXAS RED : *E. floccosum***

Variability of the signal

	Dermatophytes species	WITHIN EXPERIMENT CV average (%)	BEETWEEN EXPERIMENTS CV average (%)
Oligomix 1	MC	0.70	5.59
	TI	0.81	6.64
Oligomix 2	TR+TV	0.77	1.67
	TT	0.88	2.36
	EF	0.75	1.87

CV : Coefficient of Variation

Validation on clinical samples

Specificity and sensitivity were analyzed from 66 DNA samples extracted from mycological clinical samples (swabs rubbed on the scalp, nails, skin, hair and dander), or extracted from pure cultures of dermatophytes, isolated clinical samples and identified by macro and microscopic morphological analysis, MALDI-TOF mass spectrometer, and sequencing of the rRNA ITS2 region from a Reference Laboratory.

The species covered in this analysis are the following

- *M.canis* (MC), n= 11
- *T. interdigitale* (TI), n= 12
- *T. rubrum* (TR) + *T.violaceum* (TV), n=29
- *T. tonsurans* (TT), n= 9
- *E. floccosum* (EF), n= 5

Several tests on DNA samples not diluted or diluted have been performed; this is summed up below.
The tests were performed on CFX96.

Detection of MC and TI:

		EBX-023 DERMATOPHYTES					
		MC			**TI**		
		POSITIVE	NEGATIVE	TOTAL	POSITIVE	NEGATIVE	TOTAL
Pretest	POSITIVE	11	0	11	10	2	12
	NEGATIVE	0	35	35	0	34	34
	TOTAL	11	35	46	10	36	46

Detection of TR+TV, TT and EF:

		EBX-023 DERMATOPHYTES								
		TR+TV			**TT**			**EF**		
		POS	NEG	TOTAL	POS	NEG	TOTAL	POS	NEG	TOTAL
Pretest	POS	29	0	29	9	0	9	5	0	5
	NEG	0	28	28	0	48	48	0	52	52
	TOTAL	29	28	57	9	48	57	5	52	57

Specificity, Sensitivity and Concordance :

Targets	Specificity	Sensitivity	Concordance
Microsporum canis	> 99 %	> 99 %	> 99 % (n=46)
Trichophyton interdigitale	> 99 %	83.3 %	95.7 % (n=44/46)
*Trichophyton rubrum/violaceum**	> 99 %	> 99 %	> 99 % (n=57)
Trichophyton tonsurans	> 99 %	> 99 %	> 99 % (n=57)
Epidermophyton floccosum	> 99 %	> 99 %	> 99 % (n=57)

* cross detection between TR and TV

No cross reactivity was observed with the system of detection of :

- MC on TI, TT, EF, TR or TV samples,
- TI on MC, TT, EF, TR or TV samples,
- TV + TR on TT, EF or TI samples,
- TT on MC, TI, EF, TR or TV samples,
- EF on MC, TI, TT or TV samples,

  A late non-specific amplification for an undiluted TR sample out of six tested was observed with a Ct = 37.95; that is why the threshold of positivity for the diagnosis of EF is Ct < 37 (see table p.33).

Fungal DNA extraction was done by lysis and mechanical homogenization, and using EZ1 Mini Kit (Qiagen) following manufacturer's instructions.

Detection threshold

The analytical sensitivity was determined by serial dilutions of DNA extracted from pure cultures of dermatophytes for each target channel of this Eurobioplex. The concentration of extracted DNA was quantified by Nanodrop 2000 spectrophotometer (Thermo Scientific).

The sensitivity is expressed in pg DNA/microl and equivalent genome/microl in the table below; 0.1pg DNA corresponds to 2.5 - 3.3 equivalent genomes (Arabatzis et al., 2007).

Dermatophytes Species	**Detection threshold**	
	Concentration pg DNA/microl	**Equivalent Genome/microl**
Microsporum canis (MC)	0.55	13.75 – 18.15
Trichophyton interdigitale (TI)	0.60	15 - 20
Trichophyton rubrum (TR)	4.57	114 - 151
Trichophyton tonsurans (TT)	0.005	0.125 – 0.165
Epidermophyton floccosum (EF)	0.41	10 – 13.53

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WASTE DISPOSAL

Be in accordance with the law for the elimination of waste of clinical infectious material.

SYMBOLS

REF	Reference
LOT	Batch number
	Limits of storage temperature
	Expiration Date
	Content sufficient for « N » reactions
	Keep protected from light
	Manufacturer
	Instructions for use
CE	CE labeled product
IVD	In vitro diagnostic

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