RUO

INSTRUCTION FOR USE

Nail Panel PCR Kit

For Research Use Only

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REF MBLNAIL009

06 2024

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Document Revision History

Rev.No_Date	Revision Description
Rev.00_June 20, 2024	First Release

CONTENTS

1. INTENDED USE

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease. Furthermore, the test kit is not intended to diagnose infectious animal diseases.

The ***MarinaBiolab Nail Panel PCR Kit*** is a multiplexed qualitative Real-Time Polymerase Chain Reaction (qPCR) test intended for the simultaneous detection and identification of multiple pathogenic nucleic acids in research samples. The ***MarinaBiolab Nail Panel PCR Kit*** allows to achieve qPCR result in less than 1 hour. The test is performed to detect gene sequences of the following organisms.

Targets	
Microsporum gypseum	*Candida auris*
Microsporum canis/ audouinii	*Aspergillus niger*
Trichophyton spp	*Aspergillus flavus*
Malassezia furfur	*Aspergillus fumigatus*
Malassezia sympodialis	*Aspergillus terreus*
Candida krusei	*Epidermophyton floccosum*
Candida albicans	*Fusarium oxysporum*
Candida glabrata	*Trichosporon asahii*
Candida parapsilosis	*Trichosporon mucoides*
Controls	
Human RNase P (IC)	
Bacillus atrophaeus (EC)	

2. PRINCIPLE of the PROCEDURE

DNA target regions are amplified via real-time PCR instruments using the primer and probe sets in the kit. In the process, the probe anneals a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instruments. Probes labeled with different fluorophores are used to detect specific amplicons originating from targets and Internal Control.

PCR instruments measure these signals at the end of each amplification cycle in real time and interpret the data to provide a qualitative result for each of the above targets. A positive result for the detection of target DNA is indicated by the presence of a real-time PCR growth curve and an associated Cq (Quantification Cycle) value.

3. KIT COMPONENTS

The ***MarinaBiolab Nail Panel PCR Kit*** consists of four main components:

1. qPCR Enzyme and Buffer Mix (qPCR Master Mix)
2. Forward, Reverse and Probe Oligo Mix (NP Oligo Mix 1-8)
3. Mix of non-infectious DNA from artificial sample including targets in the table below (PC-NP)
4. DNase/RNase-Free Water (NTC)

The kit components are provided in Table 1-2.

Table 1. Kit components.

Component	Description	Quantity x Volume
		100 rxn **MBLNAIL009**
qPCR Master Mix	Ready-to-use mix for qPCR	4 x 1000 µL
NP Oligo Mix 1-8	Primers and probes complementary to specific regions of the targets in the table above	8 x 250 µL
PC-NP	Mix of non-infectious DNA from artificial sample including targets in the table above	2 x 400 µL
NTC	DNase/RNase-Free Water	2 x 400 µL

Table 2. Oligo Mix target organisms and detection channels.

Vial Name	Target	Channel
NP Oligo Mix 1	*Epidermophyton floccosum*	**FAM/Green**
	Candida krusei	**HEX/VIC/JOE/Yellow**
	Trichosporon asahii	**ROX/Texas Red/Orange**
	-	**CY5/Red**
NP Oligo Mix 2	*Candida albicans*	**FAM/Green**
	Aspergillus flavus	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	Bacillus atrophaeus (EC)	**CY5/Red**
NP Oligo Mix 3	*Aspergillus niger*	**FAM/Green**
	Candida glabrata	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	Fusarium oxysporum	**CY5/Red**

NP Oligo Mix 4	*Trichophyton spp*	**FAM/Green**
	-	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	Candida auris	**CY5/Red**
NP Oligo Mix 5	*Malassezia furfur*	**FAM/Green**
	-	**HEX/VIC/JOE/Yellow**
	Candida parapsilosis	**ROX/Texas Red/Orange**
	Aspergillus terreus	**CY5/Red**
NP Oligo Mix 6	-	**FAM/Green**
	-	**HEX/VIC/JOE/Yellow**
	Aspergillus fumigatus	**ROX/Texas Red/Orange**
	Human RNase P (IC)	**CY5/Red**
NP Oligo Mix 7	*Microsporum gypseum*	**FAM/Green**
	Microsporum canis/audouinii	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	-	**CY5/Red**
NP Oligo Mix 8	-	**FAM/Green**
	Malassezia sympodialis	**HEX/VIC/JOE/Yellow**
	-	**ROX/Texas Red/Orange**
	Trichosporon mucoides	**CY5/Red**

The oligonucleotide set targeting the human *RNase P* mRNA (Internal Control: IC) and *Bacillus atrophaeus* (External Control: EC) are used to monitor sampling, nucleic acid extraction, and inhibition of qPCR. The kit also contains negative and positive control templates for evaluating the contamination and the qPCR reagent stability, respectively.

4. EQUIPMENT and MATERIALS REQUIRED but NOT PROVIDED

- 2-8°C Refrigerator
- ≤ -20°C Freezer
- ≤ -70°C Freezer (Optional)
- Vortex mixer
- Benchtop centrifuge with rotor for 1.5 mL tubes
- Benchtop mini centrifuge with rotor for PCR strips
- Benchtop plate centrifuge
- Biological Safety Cabinet (BSC)
- PCR cabinet for PCR Setup
- Adjustable Micropipettes: 1-10, 10-100, 100-1000 µL
- Sterile DNase/RNase free micropipettes tips - Compatible with the micropipettes
- Cold tube rack for microfuge tubes (1.5/2 mL) and for PCR tubes (0.1/0.2 mL)
- Disposable, powder-free, nitrile gloves
- Disposable (preferably) laboratory coat
- Surface decontaminants - Freshly diluted 10% bleach solution (0.5% NaClO)
- Applied Biosystems QuantStudio 5, 7, and 12K with Design & Analysis software and consumables
- Bio-Rad CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/CFX384 Touch™/CFX Opus 384™ with Maestro software v1.1 and consumables
- Qiagen Rotor-Gene Q 5plex Platform with Rotor-Gene Q series software v2.1.0.9 and consumables
- Roche LightCycler 480 with software and consumables

5. WARNING and PRECAUTIONS

- The ***MarinaBiolab Nail Panel PCR Kit*** is designed for research use only and should be used by professionally trained, qualified staff only. All work should be performed using Good Laboratory Practices.
- Biological material used for extraction of nucleic acid should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).
- Biological material should be inactivated before disposal (e.g., in an autoclave). Disposables should be autoclaved or incinerated after use.
- Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol. Material used to clean spills, including gloves, should be inactivated before disposal (e.g., in an autoclave).
- Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state, and local regulations.
- Microbial contamination of the reagents while taking aliquots should be avoided. It is recommended to use sterile one-way pipettes and tips. Reagents that look cloudy or show any signs of microbial contamination must not be used.
- The kit should be stored away from nucleic acid sources and qPCR amplicons.
- Always check the expiration date on the kit. Do not use expired or incorrectly stored kit.
- The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
- Kit components should be mixed by gently shaking before use.
- A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. To prevent amplicon contamination:
    - It shall be ensured that separate work areas with their own apparatus are available.
    - Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
    - Discard used tubes/plates in a biohazard container immediately after the run has completed.
    - Avoid excessive handling of tubes/plates after test runs.
    - Change gloves after handling a used tubes/plate.

6. HANDLING, STORAGE, and STABILITY

- The ***MarinaBiolab Nail Panel PCR Kit*** is shipped on dry ice. If any component except qPCR Master Mix of the kit is not frozen on arrival, or if the outer packaging has been compromised during shipment, please contact **MarinaBiolab** or the local distributors as soon as possible.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated freezing and thawing of the kit components may result in lower detection quality. The kit can undergo up to 15 freeze/thaw cycles without affecting performance.
- When stored under the specified storage conditions, the kit is stable until the stated expiration date printed on the package. The expiration date of the kit is 12 months from date of manufacture.
- All components must be thawed at ambient temperature for a minimum of 30 minutes before use.
- It is recommended that all components should be kept on ice when setting up the assay mixes.
- The primer and probe mixes contain fluorophore labeled probes and should be protected from direct sunlight or long-term ambient light.
- Do not use expired or incorrectly stored components.

7. TEST PROCEDURE

7.1. Sample Preparation and Nucleic Acid Extraction

The sample material for the isolation of nucleic acid must be sent in appropriate cell collection systems. The performance of the kit strongly depends on the amount and quality of the extracted nucleic acid. It must be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

If the established standard method of the lab is used for nucleic acid isolation, it must be validated by the user.

For frozen samples or frozen extracted nucleic acid, only thaw the number of specimen extracts that will be tested in a single day.

Do not freeze/thaw extracted nucleic acid more than once before testing as each freeze/thaw cycle can decrease the nucleic acid quality. For optimal results, use it directly.

7.2. PCR Reaction Preparation and Processing

- Completely thaw the components at room temperature for a minimum of 30 minutes before each use.
- Place all components on ice once thawed during the whole test procedure.
- Determine the number of reactions and create the PCR plate plan.
- Include the following reactions to the plan:
    - Reactions for each test sample and extraction negative control.
    - PCR control reactions:
        - Positive Control (included in the kit)
        - Negative (No Template) Control (NTC) (included in the kit)
        - No Template Addition Control (NRC)
- Vortex and spin down briefly the components before each use.
- Combine the following components for the number of reactions required plus 10% overage to compensate for pipetting errors:

Table 3. Reaction set-up.

Reaction Mix Component	1X Reaction (µL) per well
qPCR Master Mix	5 µL
NP Oligo Mix 1-8	2.5 µL
Template Nucleic Acid	2.5 µL
Total Reaction Volume	10 µL

- Add 5 µL of qPCR Master Mix and 2.5 µL of NP Oligo Mix 1-8 into PCR tubes.
- Add 2.5 µL of the isolated sample into the individual tubes.
- The final reaction mix volume is 10 µL.
- Close the tubes, centrifuge briefly, insert tubes into the real-time PCR instrument and amplify according to the following PCR profile.

Table 4. Amplification profile.

Step	Number of Cycles	Temperature	Time	Data Collection
Initial Denaturation	1	95 °C	10 sec	**FAM/Green**, **HEX/VIC/JOE/Yellow**, **ROX/Texas Red/Orange**, **CY5/Red**
Denaturation	40	95 °C	5 sec	
Annealing/Extension		55 °C	15 sec	

8. INTERPRETATION OF RESULTS

MarinaBiolab Nail Panel PCR Kit provides a qualitative result for the presence (Detected) or absence (Not Detected) of the target genes.

8.1. Calculation of Cq Values and Instrument-Specific Requirements

Perform the following instrument settings before evaluating the results.

Table 5. Instrument-specific requirements before evaluating the results.

Instrument	Threshold Level	Other Settings
CFX96 Touch™/CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/ CFX384 Touch™/CFX Opus 384™ (Bio-Rad)	500 RFU	-
Rotor-Gene Q 5plex Platform (QIAGEN)	0.02 RFU	Dynamic Tube: Active Slope Correct: Active Outlier Removal: 0
QuantStudio™ 5, 7 and 12K (Applied Biosystems™)	Auto	-
Roche LightCycler 480 (Roche)	Auto	-

The shape of the amplification curves should be examined. If a Cq value is assigned to a sample by the instruments' software and the curve is sigmoidal, the Cq value can be used in the final evaluation. *Non-sigmoidal curves should be recorded as negative.*

The result is recorded as positive if Cq≤38 or as established by your lab.

8.2. Overall Validity of Detection

Table 6. Expected performance of controls.

Control Type	Used to Monitor	Signal	
		Target Channel	Internal/External Control Channel
Negative Control	Cross-contamination during extraction and reaction setup	-	-
No template addition	Reagent and/or environmental contamination	-	-
Positive Control	qPCR reaction setup and reagent integrity	+	+
Internal/External Control	To monitor the integrity of nucleic acid extraction and qPCR from each specimen	Not applicable	+

Before analyzing samples results, we recommend verifying if the real-time PCR test is valid. Thus, for each run, please confirm if the results for Positive and Negative controls performed as expected, according to the following criteria:

Table 7. Run validity/positive and negative control pass criteria.

Positive Control		Negative Control		Results	Recommendation
Target Channel	**Internal/External Control Channel**	**Target Channel**	**Internal/External Control Channel**		
+	+	-	-	VALID	Continue to result interpretation of samples.
Any of them is Negative		Not considered		INVALID	Contact the manufacturer, renew the reagents, and repeat the reaction.
Not considered		Any of them is Positive		INVALID	Repeat analysis, paying attention to "Warnings and Precautions" in IFU.

If any control does not perform as described above, the run is considered invalid, and the test is repeated. If the problem persists, contact the manufacturer.

If all the controls are valid, proceed to the interpretation of the results.

8.3. Interpretation of Unknown Specimen Results

The data produced by the instruments can manually be evaluated and reported using their software.

Table 8. Interpretation of unknown specimen results for DNA pathogens.

DNA Pathogens	Internal Control (*RNase P*)	External Control (*Bacillus atrophaeus*)	Results	Interpretation
Positive (+) (Cq<38)	Positive (+) (Cq<38)	Positive (+) (Cq<38)	Positive for Target	Target DNA is detected
Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Positive for Target	Target DNA is detected
Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Positive (+) (Cq<38)	Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Positive (+) (Cq<38)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Negative for Target	Target DNA is not detected
Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.
Negative (-) (Cq≥38 or N/A)	Positive (+) (Cq<38)	Negative (-) (Cq≥38 or N/A)	Invalid	Repeat test by re-extracting the sample. If the repeat result remains invalid, consider collecting a new sample.

9. ASSAY LIMITATIONS

- The ***MarinaBiolab Nail Panel PCR Kit*** is intended for use by professionally trained, qualified staff only.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Spontaneous mutations within the target sequences may result in failure to detect the target sequence. While this risk is mitigated in the test's design, if failure to detect the target is expected it is recommended to test the specimen with a different test that detects different target sequences from the target's genome.
- There is a risk of false positive results due to cross-contamination by target viruses and/or bacteria, their nucleic acids or amplified product, or from non-specific signals in the assay. Attention should be given to the handling of consumables under the Warnings and Precautions section to help minimize this risk.
- This assay is a qualitative test and does not provide a quantitative assessment of the concentration of the detected organism.
- All instruments (e.g., pipettes, real-time cyclers) must be calibrated according to the manufacturer's instructions.

10. PERFORMANCE CHARACTERISTICS

10.1. Analytical Sensitivity (Limit of Detection, LoD)

The LoD was defined as the concentration at which the test produces a positive result >95% of the time. Serial dilutions of the strains were tested and the initial tentative LoD confirmed with twenty (20) replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further twenty (20) replicates were performed at the next lower concentration until ≤95% was achieved. For nucleic acid extraction, simulated research matrix was spiked with strains and loaded onto the Automatic Nucleic Acids Extraction Instrument. The tests were carried out using the CFX96 Touch™ (Bio-Rad) Real-Time PCR system. The confirmed LoDs for the strains tested and the corresponding LoDs for the ***MarinaBiolab Nail Panel PCR Kit*** reportable targets are shown in Table 9 below.

Table 9. Summary of LoD study results.

Analyte	Isolate ID/Source	LoD Concentration (copies/mL)	Detected/Total
Microsporum gypseum	ATCC 14683	7.2E+01 copies/mL	**19/20** 95%
Microsporum audouinii	ATCC 42558	8.9E+01 copies/mL	**20/20** 100%
Microsporum canis	ATCC 36299	1.2E+02 copies/mL	**20/20** 100%
Malassezia sympodialis	ATCC 42132	6.6E+01 copies/mL	**20/20** 100%
Trichosporon asahii	ATCC 90039	6.5E+01 copies/mL	**20/20** 100%
Epidermophyton floccosum	ATCC 9646	9.8E+01 copies/mL	**20/20** 100%
Trichophyton soudanense	ATCC 24869	1.7E+02 copies/mL	**20/20** 100%
Trichophyton terrestre	ATCC 24436	1.5E+02 copies/mL	**20/20** 100%
Trichosporon mucoides	NCTC NCPF 8762	1.4E+02 copies/mL	**19/20** 95%
Trichophyton tonsurans	ATCC 56186	1.6E+02 copies/mL	**20/20** 100%
Trichophyton rubrum	Zeptometrix 0804478	8.7E+01 copies/mL	**20/20** 100%
Trichophyton violaceum	ATCC 28944	8.8E+01 copies/mL	**20/20** 100%
Trichophyton verrucosum	ATCC 28203	1.1E+02 copies/mL	**20/20** 100%

Candida krusei	ATCC 2159	6.8E+01 copies/mL	**20/20** 100%
Candida albicans	ATCC 10231	3.4E+02 copies/mL	**20/20** 100%
Candida glabrata	ATCC 90030	4.4E+01 copies/mL	**20/20** 100%
Candida parapsilosis	ATCC 22019	5.8E+01 copies/mL	**20/20** 100%
Candida auris	ATCC MYA-5003	7.2E+01 copies/mL	**19/20** 95%
Aspergillus niger	Zeptometrix 0801827	1.0E+02 copies/mL	**20/20** 100%
Aspergillus flavus	Zeptometrix 0801598	9.8E+01 copies/mL	**20/20** 100%
Aspergillus fumigatus	Zeptometrix 0801716	1.3E+02 copies/mL	**20/20** 100%
Aspergillus terreus	Zeptometrix 0801601	8.7E+01 copies/mL	**19/20** 95%
Malassezia furfur	ATCC 14521	1.2E+02 copies/mL	**20/20** 100%
Fusarium oxysporum	ATCC MYA-1198	7.8E+01 copies/mL	**19/20** 95%
Trichophyton mentagrophytes	ATCC 18748	9.8E+01 copies/mL	**20/20** 100%
Trichophyton interdigitale	ATCC 9533	1.2E+02 copies/mL	**20/20** 100%

10.2. Device Equivalence Study

Device equivalence study was carried out to observe the differences between the results to be obtained using the kit in different instruments. For this purpose, the same LoD determination study was performed again with the Bio-Rad CFX96™ Dx/CFX Opus 96™/CFX Opus 96™ Dx/CFX384 Touch™/CFX Opus 384™, Applied Biosystems QuantStudio 5, 7, and 12K, Qiagen Rotor-Gene Q 5plex Platform and Roche LightCycler 480. Similar test results were obtained with the 1x LoD concentration level of the targets in the "device equivalence study" performed with the other instruments.

10.3. Analytical Reactivity (Inclusivity)

10.3.1. In-Slico Analytical Reactivity

BLAST search of the oligonucleotides was performed on the *Microsporum gypseum, Microsporum canis/ audouinii, Trichophyton spp., Malassezia furfur, Malassezia sympodialis, Candida krusei, Candida albicans, Candida glabrata, Candida parapsilosis, Candida*

auris, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Epidermophyton floccosum, Fusarium oxysporum, Trichosporon asahii, and *Trichosporon mucoides* genome sequences available in the NCBI database, using the Primer-BLAST tool of NCBI.

The aggregated result of all in-silico analyzes performed in NCBI database is provided in Table below. The melting temperatures (Tm) of the oligonucleotide sequences with 1-base mismatch, are still higher than the annealing temperature specified in the PCR cycle parameters of the kit. Hence, the single mismatches in the sequences are not expected to affect the inclusivity of the test.

Table 10. In-silico analysis results performed in the NCBI database.

Target	Primer	Total number of target sequences	Ratio of the sequences without mismatch	Ratio of the sequences with 1 base mismatch	Ratio of the sequences with 2 base mismatches	Ratio of the sequences with 3 base mismatches
Trichophyton spp	Sense Primer	4234	96,77%	3,23%	0.00%	0.00%
Trichophyton spp	Antisense Primer	4234	96,77%	3,23%	0.00%	0.00%
Trichophyton spp	Hydrolysis Probe	4121	97,17%	2,83%	0.00%	0.00%
Microsporum canis/audouinii	Sense Primer	254	97,37%	2,63%	0.00%	0.00%
Microsporum canis/audouinii	Antisense Primer	254	97,37%	2,63%	0.00%	0.00%
Microsporum canis/audouinii	Hydrolysis Probe	250	97,14%	2,86%	0.00%	0.00%
Microsporum gypseum	Sense Primer	587	98,91%	1,09%	0.00%	0.00%
Microsporum gypseum	Antisense Primer	587	98,91%	1,09%	0.00%	0.00%
Microsporum gypseum	Hydrolysis Probe	494	98,79%	1,21%	0.00%	0.00%
Malassezia furfur	Sense Primer	302	99,67%	0,33%	0.00%	0.00%
Malassezia furfur	Antisense Primer	302	99,67%	0,33%	0.00%	0.00%
Malassezia furfur	Hydrolysis Probe	332	98,19%	1,81%	0.00%	0.00%
Malassezia sympodialis	Sense Primer	234	98,88%	1,12%	0.00%	0.00%
Malassezia sympodialis	Antisense Primer	234	98,88%	1,12%	0.00%	0.00%
Malassezia sympodialis	Hydrolysis Probe	234	97,12%	2.88%	0.00%	0.00%
Trichosporon asahii	Sense Primer	716	100,00%	0,00%	0.00%	0.00%
Trichosporon asahii	Antisense Primer	716	100,00%	0,00%	0.00%	0.00%
Trichosporon asahii	Hydrolysis Probe	725	100,00%	0,00%	0.00%	0.00%
Trichosporon mucoides	Sense Primer	24	95,83%	4,17%	0.00%	0.00%
Trichosporon mucoides	Antisense Primer	24	95,83%	4,17%	0.00%	0.00%
Trichosporon mucoides	Hydrolysis Probe	22	95,45%	4,55%	0.00%	0.00%
Epidermophyton floccosum	Sense Primer	165	99,39%	0,61%	0.00%	0.00%
Epidermophyton floccosum	Antisense Primer	165	99,39%	0,61%	0.00%	0.00%

Epidermophyton floccosum	Hydrolysis Probe	166	100,00%	0,00%	0.00%	0.00%
Candida auris	Sense Primer	501	100,00%	0,00%	0.00%	0.00%
Candida auris	Antisense Primer	501	100,00%	0,00%	0.00%	0.00%
Candida auris	Hydrolysis Probe	499	100,00%	0,00%	0.00%	0.00%
Aspergillus niger	Sense Primer	2423	94,22%	5,78%	0.00%	0.00%
Aspergillus niger	Antisense Primer	2423	94,22%	5,78%	0.00%	0.00%
Aspergillus niger	Hydrolysis Probe	2565	95,46%	4,54%	0.00%	0.00%
Aspergillus fumigatus	Sense Primer	1385	93,69%	6,31%	0.00%	0.00%
Aspergillus fumigatus	Antisense Primer	1385	93,69%	6,31%	0.00%	0.00%
Aspergillus fumigatus	Hydrolysis Probe	1355	94,98%	5,02%	0.00%	0.00%
Aspergillus flavus	Sense Primer	2762	97,25%	2,75%	0.00%	0.00%
Aspergillus flavus	Antisense Primer	2762	97,25%	2,75%	0.00%	0.00%
Aspergillus flavus	Hydrolysis Probe	2898	95,41%	4,59%	0.00%	0.00%
Aspergillus terreus	Sense Primer	1567	97,77%	2,23%	0.00%	0.00%
Aspergillus terreus	Antisense Primer	1567	97,77%	2,23%	0.00%	0.00%
Aspergillus terreus	Hydrolysis Probe	1445	98,41%	1,59%	0.00%	0.00%
Fusarium oxysporum	Sense Primer	2687	98.22%	1.78%	0.00%	0.00%
Fusarium oxysporum	Antisense Primer	2687	98.22%	1.78%	0.00%	0.00%
Fusarium oxysporum	Hydrolysis Probe	2790	97.99%	2.01%	0.00%	0.00%
Candida krusei	Sense Primer	1.415	100%	0.00%	0.00%	0.00%
Candida krusei	Antisense Primer	1.415	100%	0.00%	0.00%	0.00%
Candida krusei	Hydrolysis Probe	1.415	100%	0.00%	0.00%	0.00%
Candida albicans	Sense Primer	3.629	99.69%	0.31%	0.00%	0.00%
Candida albicans	Antisense Primer	3.728	98.85%	2.25%	0.00%	0.00%
Candida albicans	Hydrolysis Probe	3.728	98.52%	2.48%	0.00%	0.00%
Candida parapsilosis	Sense Primer	2.559	99.74%	0.26%	0.00%	0.00%
Candida parapsilosis	Antisense Primer	2.463	100%	0.00%	0.00%	0.00%
Candida parapsilosis	Hydrolysis Probe	2.463	100%	0.00%	0.00%	0.00%
Candida glabrata	Sense Primer	763	100%	0.00%	0.00%	0.00%
Candida glabrata	Antisense Primer	1.111	99.20%	0.80%	0.00%	0.00%
Candida glabrata	Hydrolysis Probe	1.111	99.64%	0.36%	0.00%	0.00%

10.3.2. Wet-Test Analytical Reactivity

The analytical reactivity (inclusivity) of the ***MarinaBiolab Nail Panel PCR Kit*** was demonstrated with a comprehensive panel representing temporal, evolutionary, and geographic diversity for each of the target organisms.

Each sample was tested with the ***MarinaBiolab Nail Panel PCR Kit*** in triplicate at an initial concentration 3-fold higher than the LoD determined for each analyte. In cases where the expected targets were not detected in one or more replicates, concentrations at a 3-fold higher level were evaluated.

The individual strains and concentrations at which positive test results were obtained for all three (3) replicates are presented by target organism in Table 11 below.

Table 11. Results of the wet inclusivity test.

Variant/Type/Subtype/Lineage/Genotype/Species	Isolate ID/Source	xLoD Detected
Microsporum gypseum	ATCC 14683	1x
Microsporum audouinii	ATCC 42558	1x
Microsporum canis	ATCC 36299	1x
Malassezia sympodialis	ATCC 42132	1x
Trichosporon asahii	ATCC 90039	1x
Epidermophyton floccosum	ATCC 9646	1x
Trichophyton soudanense	ATCC 24869	1x
Trichophyton terrestre	ATCC 24436	1x
Trichosporon mucoides	NCTC NCPF 8762	1x
Trichophyton tonsurans	ATCC 56186	1x
Trichophyton rubrum	Zeptometrix 0804478	1x
Trichophyton violaceum	ATCC 28944	1x
Trichophyton verrucosum	ATCC 28203	1x
Candida krusei	ATCC 2159	1x
Candida albicans	ATCC 10231	1x
Candida glabrata	ATCC 90030	1x
Candida parapsilosis	ATCC 22019	1x
Candida auris	ATCC MYA-5003	1x
Aspergillus niger	Zeptometrix 0801827	1x
Aspergillus flavus	Zeptometrix 0801598	1x
Aspergillus fumigatus	Zeptometrix 0801716	1x
Aspergillus terreus	Zeptometrix 0801601	1x

Malassezia furfur	ATCC 14521	1x
Fusarium oxysporum	ATCC MYA-1198	1x
Trichophyton mentagrophytes	ATCC 18748	1x
Trichophyton interdigitale	ATCC 9533	1x

10.4. Analytical Specificity (Exclusivity)

10.4.1. In-Slico Analytical Specificity

Primers and probes intended for a target sequence may also attach to similar sequences if they closely match or differ by only a few base pairs from the non-targeted sequence. To ensure specificity to the target amplicon sequence, it's essential to screen the primers and probe against the reference database transcript or genome database for the intended templates, as well as any databases containing potential contaminating templates.

Table 12. The results of On-Panel and Off-Panel organisms tested for cross-reactivity.

On-Panel/Off-Panel	Name of the organism	Cross Reactivity*		
		Forward	Probe	Reverse
On-Panel	*Microsporum gypseum*	None	None	None
On-Panel	*Microsporum audouinii*	None	None	None
On-Panel	*Microsporum canis*	None	None	None
On-Panel	*Malassezia sympodialis*	None	None	None
On-Panel	*Trichosporon asahii*	None	None	None
On-Panel	*Epidermophyton floccosum*	None	None	None
On-Panel	*Trichophyton soudanense*	None	None	None
On-Panel	*Trichophyton terrestre*	None	None	None
On-Panel	*Trichosporon mucoides*	None	None	None
On-Panel	*Trichophyton tonsurans*	None	None	None
On-Panel	*Trichophyton rubrum*	None	None	None
On-Panel	*Trichophyton violaceum*	None	None	None
On-Panel	*Trichophyton verrucosum*	None	None	None
On-Panel	*Candida krusei*	None	None	None
On-Panel	*Candida albicans*	None	None	None
On-Panel	*Candida glabrata*	None	None	None
On-Panel	*Candida parapsilosis*	None	None	None
On-Panel	*Candida auris*	None	None	None

On-Panel	*Aspergillus niger*	None	None	None
On-Panel	*Aspergillus flavus*	None	None	None
On-Panel	*Aspergillus fumigatus*	None	None	None
On-Panel	*Aspergillus terreus*	None	None	None
On-Panel	*Malassezia furfur*	None	None	None
On-Panel	*Fusarium oxysporum*	None	None	None
On-Panel	*Trichophyton mentagrophytes*	None	None	None
On-Panel	*Trichophyton interdigitale*	None	None	None
Off-Panel	*Acremonium potronii*	None	None	None
Off-Panel	*Neofusicoccum mangiferae*	None	None	None
Off-Panel	*Scopulariopsis brevicaulis*	None	None	None
Off-Panel	*Aspergillus versicolor*	None	None	None
Off-Panel	*Acremonium strictum*	None	None	None
Off-Panel	*Sarcoptes scabiei*	None	None	None
Off-Panel	*Pseudomonas aeruginosa*	None	None	None
Off-Panel	*Candida lusitaniae*	None	None	None
Off-Panel	*Candida dubliniensis*	None	None	None
Off-Panel	*Bartonella quintana*	None	None	None
Off-Panel	*Bartonella henselea*	None	None	None
Off-Panel	*Aspergillus clavatus*	None	None	None
Off-Panel	*Aspergillus nidulans*	None	None	None
Off-Panel	*Candida guilliermondi*	None	None	None
Off-Panel	*Aspergillus oryzae*	None	None	None
Off-Panel	*Candida humilis*	None	None	None
Off-Panel	*Fusarium solani*	None	None	None
Off-Panel	*Fusarium onychomycoses*	None	None	None
Off-Panel	*Epidermophyton stockdaleae*	None	None	None
Off-Panel	*Scopulariopsis asperula*	None	None	None
Off-Panel	*Scopulariopsis brumptii*	None	None	None
Off-Panel	*Scopulariopsis asperula*	None	None	None
Off-Panel	*Paecilomyces variotii*	None	None	None
Off-Panel	*Acremonium acutatum*	None	None	None

Off-Panel	*Acremonium chilense*	None	None	None
Off-Panel	*Microsporum fulvum*	None	None	None
Off-Panel	*Microsporum nanum*	None	None	None
Off-Panel	*Microsporum gallinae*	None	None	None
Off-Panel	*Malassezia dermatis*	None	None	None
Off-Panel	*Malassezia japonica*	None	None	None
Off-Panel	*Malassezia vespertilionis*	None	None	None
Off-Panel	*Malassezia caprae*	None	None	None

* Homology should be <80% between the cross-reactivity microorganisms and the test primers/ probe(s).

** In silico sequence analysis indicates the potential for cross-reactivity of Bordetella pertussis with certain strains of Bordetella bronchiseptica.

10.4.2. Wet-Test Analytical Specificity

The potential for non-specific amplification by assays for detection of analytes was evaluated by testing high concentrations of organisms or nucleic acids with the ***MarinaBiolab Nail Panel PCR Kit***. On-panel organisms were tested to assess the potential for intra-panel cross-reactivity, and off-panel organisms were tested to evaluate panel specificity. Off-panel organisms included normal flora and pathogens that may be present in specimens as well as near-neighbors or species genetically related to the organisms detected by the ***MarinaBiolab Nail Panel PCR Kit***. The concentration of organism tested (in triplicate) was at least 1.0E+06 CFU/mL for bacteria, fungi and parasite, and at least 1.0E+05 unit/mL for viruses. For the few organisms of interest that were not available for laboratory testing, results of in silico analysis of the organism's whole genome sequences are indicated. The on-panel and off-panel organisms tested are shown in Table 13 and Table 14.

Table 13. On-Panel organisms tested for evaluation of ***MarinaBiolab Nail Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Microsporum gypseum	ATCC 14683	None
Microsporum audouinii	ATCC 42558	None
Microsporum canis	ATCC 36299	None
Malassezia sympodialis	ATCC 42132	None
Trichosporon asahii	ATCC 90039	None
Epidermophyton floccosum	ATCC 9646	None
Trichophyton soudanense	ATCC 24869	None
Trichophyton terrestre	ATCC 24436	None
Trichosporon mucoides	NCTC NCPF 8762	None
Trichophyton tonsurans	ATCC 56186	None
Trichophyton rubrum	Zeptometrix 0804478	None

Trichophyton violaceum	ATCC 28944	None
Trichophyton verrucosum	ATCC 28203	None
Candida krusei	ATCC 2159	None
Candida albicans	ATCC 10231	None
Candida glabrata	ATCC 90030	None
Candida parapsilosis	ATCC 22019	None
Candida auris	ATCC MYA-5003	None
Aspergillus niger	Zeptometrix 0801827	None
Aspergillus flavus	Zeptometrix 0801598	None
Aspergillus fumigatus	Zeptometrix 0801716	None
Aspergillus terreus	Zeptometrix 0801601	None
Malassezia furfur	ATCC 14521	None
Fusarium oxysporum	ATCC MYA-1198	None
Trichophyton mentagrophytes	ATCC 18748	None
Trichophyton interdigitale	ATCC 9533	None

Table 14. Off-Panel organisms were tested for evaluation of ***MarinaBiolab Nail Panel PCR Kit*** analytical specificity.

Organism	Isolate ID/Source	Cross Reactivity Detected
Scopulariopsis brevicaulis	ATCC 36840	None
Aspergillus versicolor	Zeptometrix 0801822	None
Pseudomonas aeruginosa	Zeptometrix 0801908	None
Candida lusitaniae	Zeptometrix 0801603	None
Candida dubliniensis	Zeptometrix 0801915	None
Bartonella quintana	Zeptometrix 0804360	None
Bartonella henselea	Zeptometrix 0804359	None
Aspergillus clavatus	ATCC 20062	None
Aspergillus nidulans	ATCC 10074	None
Candida guilliermondi	Zeptometrix 0801602	None
Aspergillus oryzae	ATCC 1011	None
Candida humilis	ATCC 22992	None
Fusarium solani	Zeptometrix 0801806	None
Scopulariopsis asperula	ATCC 58360	None

Paecilomyces variotii	ATCC 26820	None
Acremonium acutatum	ATCC 32209	None
Microsporum gallinae	ATCC 22242	None
Malassezia dermatis	ATCC MYA-4955	None

10.5. Interferences

The ability of endogenous or exogenous substances that could be present in research samples (or introduced during sample collection and handling) to interfere with accurate detection of analytes was evaluated with select direct testing on the ***MarinaBiolab Nail Panel PCR Kit*** and extrapolated from the interference evaluation of the ***MarinaBiolab Nail Panel PCR Kit***.

Potentially interfering substances were evaluated using contrived samples spiked with substance. Results from samples containing a substance were compared to results from control samples without substance. The substances tested included endogenous substances that may be found in sample at normal or elevated levels (e.g., blood, mucus/mucin, human genomic DNA), various commensal or infectious microorganisms, medications, washes or topical applications, various swabs and transport media for sample collection, and substances used to clean, decontaminate, or disinfect work areas. Each substance was added to contrived samples containing representative organisms at concentrations near (3x) LoD. The concentration of substance added to the samples was equal to or greater than the highest level expected to be in research sample and each sample was tested in triplicate.

None of the substances were shown to interfere with the ***MarinaBiolab Nail Panel PCR Kit***.

Table 15. Evaluation of potentially interfering substances on the ***MarinaBiolab Nail Panel PCR Kit***.

Substance Tested	Concentration Tested	Observed Interference
Endogenous Substances		
Whole Blood	10% v/v	No Interference
Human serum	5% v/v	No Interference
Competitive Microorganisms		
Microsporum gypseum	1.0E+06 CFU/mL	No Interference
Microsporum audouinii	1.0E+06 CFU/mL	No Interference
Microsporum canis	1.0E+06 CFU/mL	No Interference
Malassezia sympodialis	1.0E+06 CFU/mL	No Interference
Trichosporon asahii	1.0E+06 CFU/mL	No Interference
Epidermophyton floccosum	1.0E+06 CFU/mL	No Interference
Trichophyton soudanense	1.0E+06 CFU/mL	No Interference
Trichophyton terrestre	1.0E+06 CFU/mL	No Interference
Trichosporon mucoides	1.0E+06 CFU/mL	No Interference
Trichophyton tonsurans	1.0E+06 CFU/mL	No Interference

Trichophyton rubrum	1.0E+06 CFU/mL	No Interference
Trichophyton violaceum	1.0E+06 CFU/mL	No Interference
Trichophyton verrucosum	1.0E+06 CFU/mL	No Interference
Candida krusei	1.0E+06 CFU/mL	No Interference
Candida albicans	1.0E+06 CFU/mL	No Interference
Candida glabrata	1.0E+06 CFU/mL	No Interference
Candida parapsilosis	1.0E+06 CFU/mL	No Interference
Candida auris	1.0E+06 CFU/mL	No Interference
Aspergillus niger	1.0E+06 CFU/mL	No Interference
Aspergillus flavus	1.0E+06 CFU/mL	No Interference
Aspergillus fumigatus	1.0E+06 CFU/mL	No Interference
Aspergillus terreus	1.0E+06 CFU/mL	No Interference
Malassezia furfur	1.0E+06 CFU/mL	No Interference
Malassezia globose	1.0E+06 CFU/mL	No Interference
Malassezia restricta	1.0E+06 CFU/mL	No Interference
Fusarium oxysporum	1.0E+06 CFU/mL	No Interference
Trichophyton mentagrophytes	1.0E+06 CFU/mL	No Interference
Trichophyton interdigitale	1.0E+06 CFU/mL	No Interference
Exogenous Substances		
Moisturizing Hand Cream	5% v/v	No Interference
Specimen Collection Materials		
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference

11. TROUBLESHOOTING

Problem	Cause	Solution
Target-specific and/or IC signals are detected in the Negative Control well.	Contamination from the environment, contamination of extraction and/or qPCR reagents, or well-to-well cross contamination. The signal is not true target amplification, but background curves generated by the software of the qPCR instrument.	Repeat the qPCR with new reagents. Follow the general rules of GLP in a PCR lab (e.g., Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Ensure that filters tips are used during the procedure and changed between samples.). It is recommended to set up the qPCR reactions in a separate area, where no RNA/DNA is handled and with equipment designated for pre-PCR activities. Ignore the Cq value of NTC if the amplification curve looks not real but background noise. If the problem persists, contact Technical Support.
No IC signal is detected, but target-specific signal is detected in sample wells.	A high copy number of target nucleic acid exists in samples, resulting in preferential amplification of the target-specific nucleic acid.	No action is required. The result is considered positive.
The Positive Control did not meet the criteria set for acceptable values of the kit. The assay is invalid.	Positive Control was not stored at the recommended conditions. Kit shelf-life expired.	Check the kit label for storage conditions and expiration date. Replace the Positive Control. Use a new kit if necessary.
High Cq values observed for repeated samples.	Frozen samples were not mixed properly after thawing. Degraded nucleic acids.	Make sure, thaw frozen samples with mild agitation to ensure thorough mixing. Ensure that samples are stored correctly and not subjected to multiple freeze-thaw cycles
Target-specific and/or IC signal detected after 38 Cycles in Positive Control.	Incorrect qPCR set-up or the kit reagents may have been compromised (e.g., improper storage or more than 15 freeze-thaw cycles).	Replace the control. If the problem persists, contact Technical Support.
No target-specific and IC signal is detected in sample wells.	Sampling, extraction, or inhibition problem.	Dilute the nucleic acid isolate 1/10 and repeat the qPCR. If the diluted sample does not give a positive result in the IC channel, request for a new sample and repeat the NA extraction. Repeat the NA extraction and the qPCR. Request for a new sample, repeat the NA extraction and the qPCR. If the problem persists, contact Technical Support.

12. EXPLANATION of SYMBOLS

Symbol	Title of Symbol	Symbol	Title of Symbol
	Research Use Only		Use-by date
	Manufacturer		Batch code
	Negative control		Non-sterile
	Positive control		Consult instructions for use or consult electronic instructions for use
	Control		Caution
	Temperature limit		Catalogue number
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Keep dry		Keep upright
	Contains sufficient for <n> tests		Protect from heat and radioactive sources

Custom care and technical support

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e-mail customer care: accounting@marinabiolab.com

e-mail Technical Support: rd@marinabiolab.com

MarinaBiolab LTD.

Address: Hürriyet Mah. Erler So. No: 4/1A Nexus No: 13 Kartal/Istanbul/Turkey

For research use only (RUO)! Not for use in diagnostic procedures.