

# MucorGenius®

## Instructions For Use Version 1.2E

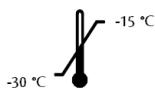


For in vitro diagnostic use.

For use with LightCycler 480® II (Roche), Rotor-Gene® Q (Qiagen), CFX96™ (Bio-Rad), Quantstudio 5 (ThermoFisher Scientific) and Magnetic Induction Cycler® (Mic qPCR cycler; Bio molecular systems) instruments



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## 1 Summary and background

Mucormycosis is associated with high mortality rates in patients 'at risk' such as hematopoietic stem cell transplant recipients, solid organ transplant, hematological malignancy, and uncontrolled diabetes mellitus. Clinically and radiographically, mucormycosis is often indistinguishable from other invasive mold infections such as aspergillosis and remains difficult to diagnose. Coinfection with *Aspergillus* species may be frequent. However, Mucorales species are not susceptible to voriconazole, which is often used as a first-line treatment in aspergillosis. Thus, early specific diagnosis and prompt therapeutic intervention with active antifungal treatment such as amphotericin B are essential for improving the outcome of mucormycosis. The increasing incidence of mucormycosis is probably related to the increase in the number of high-risk patients, particularly in those with an underlying hematological malignancy. The most common genera in invasive mucormycosis are *Rhizopus*, *Rhizomucor*, *Lichtheimia* and *Mucor*, accounting for 90% of all cases. Other genera (*Cunninghamella*, *Apophysomyces*, *Saksenaea*, *Cokeromyces*, *Actinomucor* and *Syncephalastrum*) species are individually responsible for <1% to 5% of reported cases of mucormycosis.

Currently, diagnosis is done by histopathological examination and positive culture of a Mucorales species. Real-time PCR is more often used to confirm a mucormycosis diagnosis in respiratory samples, biopsy samples, formalin-fixed, paraffin embedded tissue samples (FFPE) in cases of culture-negative invasive mold infections. A molecular diagnostic approach, using the MucorGenius® real-time PCR kit, detecting circulating DNA in serum from Mucorales in at-risk patients, may help to diagnose invasive mucormycosis more quickly and to introduce directed therapy earlier and eventually monitor treatment.

The MucorGenius® PCR can be used in parallel with the AsperGenius® Species multiplex PCR due to the same PCR protocol.

## 2 Intended use

The MucorGenius® multiplex real-time PCR kit (PN-700) is a real-time PCR kit designed to detect DNA of *Mucorales* species including *Rhizopus* spp, *Mucor* spp, *Lichtheimia* spp, *Cunninghamella* spp and *Rhizomucor* spp.

The input sample is total nucleic acid extracted and purified from Bronchoalveolar lavage (BAL), tracheal aspiration, sputum, pleural fluid, paraffin embedded tissue samples (FFPE), biopsy and serum samples.

The MucorGenius® real-time PCR kit (PN-700) is validated for use with the NucliSENS® easyMAG® (bioMérieux) and QIAamp® MinElute® Virus Spin kit (Qiagen) for nucleic acid extraction from BAL, tracheal aspiration, sputum, pleural fluid and serum samples.

The MucorGenius® real-time PCR assay aids in the diagnosis of mucormycosis in immunocompromised patients when used in combination with other clinical and laboratory findings. Negative results do not necessarily indicate absence of (fungal) infection. Negative results should not be used as the sole basis for diagnosis, therapy, or other treatment

decisions. Positive results do not exclude co-infection with other pathogens. The pathogen(s) detected may not be the definite cause of disease. Other laboratory testing and assessment of clinical presentation must be included in the final diagnosis. The product is for use by laboratory professionals only.

### 3 Principle of the test

The MucorGenius® real-time PCR assay is designed for detection of Mucorales DNA in clinical samples. The PCR assay is composed of ready to use optimized mixtures of target specific primers and probes for the detection of Mucorales DNA. The MucorGenius® real-time PCR assay is based on real-time PCR technology which is enabled by fluorescent probes present in the mixtures. Detection is performed during amplification on a real-time PCR instrument, capable of detection of fluorescence in different detection channels.

### 4 Products and targets

An overview of the MucorGenius® real-time PCR assay and its corresponding targets can be found in table 1. The Universal Colorcomp kit (PN-501) is available for LC480 II end-users.

**Table 1: MucorGenius® real-time PCR assay (PN-700)**

PN-700	MucorGenius® targets
	28S rRNA gene
	M13 phage (Internal Control)

### 5 Materials provided

The following materials are included in the MucorGenius® PCR kit (PN-700; table 2). The kit is suitable for 25 reactions.

**Table 2. Materials provided in the MucorGenius® PCR kit (PN-700)**

Components	Component number	Volume (µl)	Color of screw cap
MucorGenius® PCR mix	CNP2016A	>250	Amber
Taq polymerase	CNP2010B	>25	Purple
Dilution Buffer	CNP0012B	>950	Transparent
Internal Control	CNP2043A	>500	Black
MucorGenius® positive control	CNP2017A	>125	White
<i>MucorGenius® - Instructions for Use (English)*</i>			
<i>Material Safety Data Sheets (MSDS)#</i>			
<i>Certificate of analysis (CoA)</i>			

\*for other language versions, please contact PathoNostics or its local distributor

# On request

## 6 Materials required but not provided

The MucorGenius® PCR assay is suitable for real-time PCR instruments which have a minimum of 4 different fluorescent detection channels. The MucorGenius® PCR assay is validated on the LightCycler® 480 II (LC480 II; Roche), Rotor-Gene® Q (RGQ; QIAGEN), CFX96™ (Bio-Rad), Quantstudio® 5 (QS5; ThermoFisher scientific) and Magnetic Induction Cycler (Mic; Bio molecular systems) in combination with the NucliSENS® EasyMag® (bioMérieux) DNA-extraction system and QIAamp® MinElute® Virus Spin kit (Qiagen). Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations / quality guidelines. The materials listed below are required for the performance of the MucorGenius® protocol, but are not provided.

### Reagents

- DNA extraction components: NucliSENS® EasyMag® extraction reagents or QIAamp® MinElute® Virus Spin kit (Cat. No. 57704; Qiagen)
- DNase free/sterile PCR grade water (negative control)
- Dithiothreitol (DTT); (Thermoscientific product code #R0861)

### Consumables

- Disposable tips containing hydrophobic filters
- Sterile DNase-free 1.5 ml vials (Eppendorf tubes)
- Suitable DNase-free 0.1 or 0.2 ml PCR tubes (QIAGEN®, product code 981103 or ThermoFisher, product code AB-0620) for use of RGQ instruments
- Suitable multiwell 96 plates (Roche, product code 04 729 692 001) for use of LC480 II instruments
- DNase-free Mic tubes and caps (BMS, ref 60653) for use on the Mic qPCR cycler
- Hard-shell® PCR plates 96-well, thin wall (Bio-Rad, Hsp9655) for use on the CFX96™
- MicroAmp Fast Optical 96-well reaction plate 0.1 ml (Applied Biosystems, ref 4346906) for use on the QS5

### Equipment

- Adjustable pipettes: 0.1-2 µl, 2-20 µl, 20-200 µl, 200-1000 µl
- Tube rack for 1.5 ml vials (Eppendorf tubes)
- Cooling block (1.5 ml) or ice (for taq polymerase)
- PCR cooling block or ice for PCR reaction tubes and 96-well plates
- Vortex mixer
- Benchtop centrifuge with a rotor for 1.5 ml tubes
- Centrifuge suitable for PCR plates
- Calibrated LightCycler® 480 II, Rotor-Gene® Q, CFX96™, Quantstudio® 5 or Mic qPCR instruments
- NucliSENS® EasyMag® extraction system

## 7 General precautions

Performing lab activities should always be done in compliance with general safety regulations. For more information on chemicals, consult the appropriate material safety data sheets (MSDS), which is part of the kit insert.

The following precautions should be taken to both avoid contamination and allow optimal performance and reproducibility of the assays:

- The PCR assay should only be performed by qualified laboratory personnel.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Physically separate the workplaces as outlined in table 3.
- Use **disposable tips** containing hydrophobic **filters** to prevent cross-contamination.
- Use DNase-free PCR vials.
- Thaw **DNA samples** always on ice and keep them on **ice** or on a **cooling block**.
- Keep **enzymes** always on **ice** or on a **cooling block** when taken out of the freezer. Handle enzymes with care and mix very gently.
- When thawed, **spin down the reagents** for 5 seconds in a centrifuge and mix by gently pipetting up and down.
- The cycling program should be programmed in the real-time PCR instrument before performing the assay.
- Spin down the PCR mixtures shortly in the PCR plate before transferring to the real-time cycler (not necessary for RGQ).
- Do not open the PCR vials/plates after PCR amplification.

## 8 Reagent storage and handling

The components of the MucorGenius® kits should be stored in the dark at -15 °C to -30 °C and are stable until the expiry date which is stated on the label. Repeated thawing and freezing should be avoided (10x). To avoid cross-contamination, we recommend performing the experimental activities into three separate areas and store the controls in a separate room/location (table 3).

**Table 3. Handling procedures in different areas**

Location	Handling
Room 1	Storage PCR mix, Taq polymerase, dilution buffer Preparation real-time PCR mix
Room 2*	Storage IC and PC DNA extraction from samples Adding DNA-extracts to the real-time PCR mix Adding PC to the real-time PCR mix
Room 3	Real-time PCR reaction

\*It is recommended to perform all activities in laminar air flow safety cabinet II in order to minimize the chance of cross-contamination.

## 9 Sample storage and handling

*Mucorales* spp detection depends on the collection of high-quality specimens, their rapid transport to the laboratory, and appropriate storage before laboratory testing. Bronchoalveolar lavage (BAL), tracheal aspiration, sputum, pleural fluid, paraffin embedded tissue samples (FFPE), biopsy and serum samples can be used for the detection of *Mucorales* DNA.

Samples should be transported to the laboratory as soon as possible and should preferably be processed directly or stored at -20 °C or -70 °C upon further processing. Multiple freeze-thaw cycles should be avoided.

## 10 Nucleic acid extraction and preparation

It is recommended to perform the nucleic acid extraction and preparation including lysis in a laminar flow safety cabinet II. The extraction procedure can be continued on a workbench once the sample is lysed. It is advisable to decontaminate the laminar airflow cabinet after use with an appropriate decontamination solution and running the UV light for 30 minutes.

### 10.1 BAL/respiratory samples

It is strongly recommended to use 1 ml BAL (or other respiratory sample) for the DNA extraction and an elution volume of 50 µl. A specific DNA extraction protocol was developed for respiratory samples. Use sterile or decontaminated materials and solutions for all steps in the process.

The procedure includes the application of materials and reagents from bioMérieux for DNA extraction using the NucliSENS® EasyMag®.

- I. Vortex the respiratory sample and add 100 µl of 1 M DTT to 1 ml (final concentration 0,09 M DTT) when the sample is viscous, adjust according to the amount of material
  - ❖ Incubate the samples for 15 min at 37 °C
- II. Prepare the EasyMag® according to the manufacturer's instructions
  - ❖ Select 1 ml as input volume and 50 µl as elution volume
  - ❖ Select the generic protocol
  - ❖ Select the onboard lysis protocol
  - ❖ Thaw the internal control
- III. Transfer 1 ml sample to the EasyMag® tray
- IV. Vortex the internal control gently and add 5 µl to the sample in the EasyMag® tray
- V. Start the onboard lysis (the EasyMag® will dispense the lysisbuffer and incubates for 10 minutes
  - ❖ Prepare the magnetic silica bead solution
- VI. After lysis, add the prepared magnetic silica beads to the lysis buffer with sample and resuspend
  - ❖ Start the DNA-extraction on the EasyMag®
  - ❖ After 34-40 min, transfer the eluted DNA to DNase-free tubes

## 10.2 Serum

Due to the low fungal load it is recommended to use 1 ml of serum for the DNA extraction and an elution volume of 50 µl. Use sterile or decontaminated materials and solutions for all steps in the process. The procedure includes the application of materials and reagents from bioMérieux for DNA extraction using the NucliSENS® EasyMag®. Follow steps II to VI as described in section 10.1.

## 10.3 Other sample types

For DNA-extraction of FFPE material or biopsies contact PathoNostics or your local distributor for dedicated protocols.

## 10.4 Other DNA-extraction platforms

Although other DNA extraction platforms **are not validated**, performance evaluation studies have been performed. It is important to note that the extraction efficiency varies between DNA-extraction platforms and the DNA yield can be different. Contact PathoNostics for experience with the particular DNA extraction platform.

## 10.5 Controls

### Internal Control (IC)

The IC in the kit is supplied as a M13 bacteriophage solution and is added to discriminate between true negative samples and false negative samples which can be a result of nucleic acid degradation, PCR inhibition or test failure.

The volume of IC to be spiked into the lysed BAL sample is 5 µl. When the elution volume (50 µl) is changed, the amount of spiked IC has to be changed accordingly. The amount of spiked IC is irrespective of the initial volume of the sample.

### Positive Control (PC)

The PC in the MucorGenius® PCR kit consists of a synthetic DNA fragment and covers the target region which is detected in the assay. The PC is handled like a normal nucleic acid extract and controls for a correct PCR procedure. It is not necessary to apply the PC to the nucleic acid extraction procedure.

## 11 Real-time PCR instrument settings

The MucorGenius® real-time PCR kit is validated on real-time instruments LC480 II, RGQ, CFX96, QS5 and Mic qPCR cycler utilizing four different detection channels.

**For all mentioned real-time instruments, a PCR protocol template file and specific installation guide is available. This can be requested at PathoNostics or your local distributor.**

### Filter settings

The filter settings of all real-time PCR instruments are listed in table 4. These settings must be used to run the MucorGenius® real-time PCR assay successfully. Programming of the instruments should be carried out according to the user's manual.

**Note:** Within the same experiment both MucorGenius® and AsperGenius® Species multiplex real-time PCR assays can be applied using detection in four different channels. Consult the AsperGenius® Species IFU to activate all four detection channels.

**Table 4. Filter settings for optimal detection of MucorGenius® probes.** These channels are required when running MucorGenius® without AsperGenius®

MucorGenius®	Rotor-Gene (nm)		LC480 II (nm)		CFX96	QS5	Mic qPCR
	Source	Detector	Source	Detector			
<i>Mucorales</i> spp.	530	555	533	580	HEX	520-558 550-586	Yellow
IC	625	660	618	660	CY5	640-682	Red

### Real-time PCR cycling program: general information

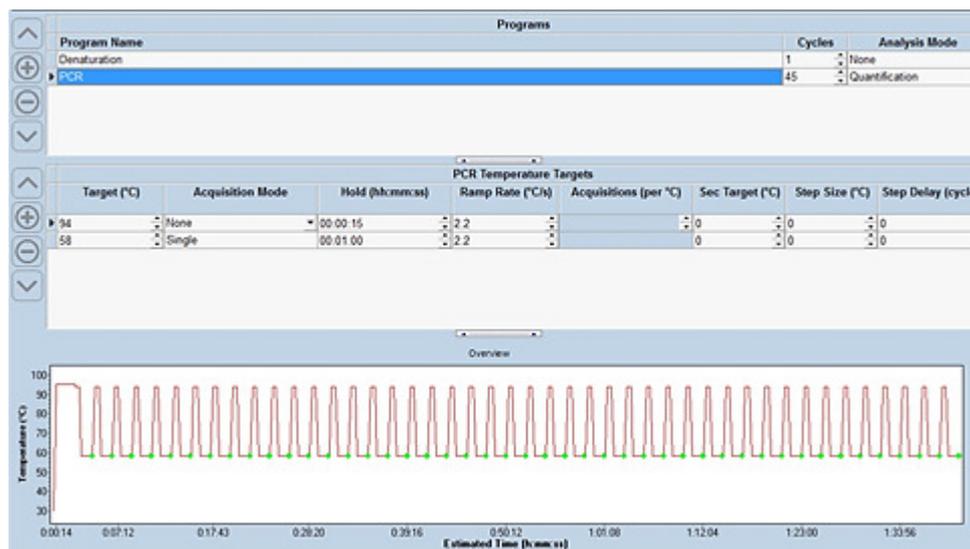
The cycling program is shown in table 5. An example of LC480 II programming is shown in figure 1.

The amplification program takes approximately 100 minutes and is identical to the amplification program of the AsperGenius® Species multiplex (PN-001), with the exception of the detection format.

**Table 5. Real-time PCR cycling program**

Time	Temperature	Function
2 min	95 °C	Taq activation/initial denaturation
15 sec	94 °C	Denaturation
60 sec	58 °C <sup>#</sup>	Annealing and extension
		} PCR 45 cycles

<sup>#</sup>activate fluorescent detection according to table 4.



**Figure 1.** Screen for the amplification in the LC480II software. Select quantification in the analysis mode and single in the acquisition mode for detection during cycling.

## 12 Procedure

### Recommendations

- Thaw template DNA (if frozen) and all reagents, and keep on ice.
- Keep the MucorGenius® Taq polymerase always on a cooling block or on ice
- Prepare the mix for a slightly larger amount than the required reactions to compensate for pipetting losses (eg. 1 additional reaction for 9 samples).

#### 12.1 Room 1: preparation of the real-time PCR mix

The real-time PCR reaction is performed in a final volume of 25 µl.

- Prepare the real-time PCR mix according to table 6.
- Mix the real-time PCR mix gently but thoroughly and dispense 20 µl of the mix into a PCR vial or well from a plate. Keep the PCR vials or plates on ice or on a cooling block during preparation.

**Table 6. Real-time PCR mix for MucorGenius®**

Component	Volume/reaction (µl)	Volume for 9 samples + 1 (µl)
MucorGenius® PCR mix	10	100
Taq polymerase	1	10
Dilution buffer	9	90
Total Volume	20	200

\*Keep on ice or cooling block

## 12.2 Room 2: Adding DNA to the PCR mixture

- Add 5 µl of extracted DNA from the sample (containing IC) to the dispensed real-time PCR mix
- For the NTC reactions: add 5 µl dilution buffer to the real-time PCR mix
- PC reactions: add 5 µl MucorGenius® positive control to the real-time PCR mix
- Close the PCR vials or seal the real-time PCR plate and spin down briefly (only necessary for a real-time PCR plate). Try to avoid air bubbles in the plate.

## 12.3 Room 3: Start the real-time PCR instrument

- Place the PCR vials or real-time PCR plate in the real-time PCR instrument
- Select the real-time cycler program and start the run (the real-time PCR cycling program is shown in table 5).

# 13 Data analysis

## 13.1 Cycle threshold ( $C_t$ ) methods

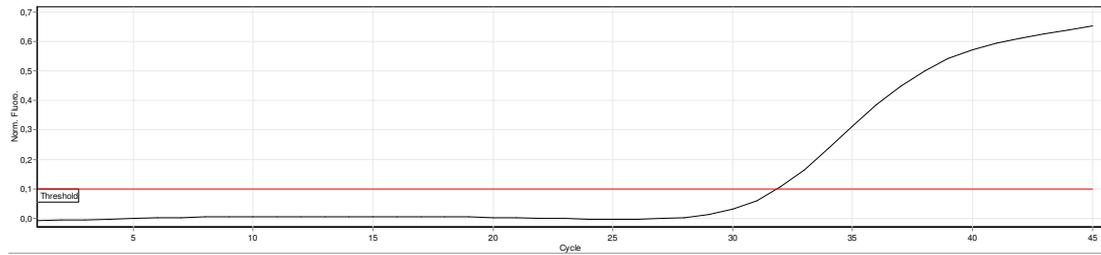
Results of the MucorGenius® real-time PCR assay are reported as  $C_t$  (cycle threshold) values depending on the particular real-time PCR instrument used and the  $C_t$  calculation method. Before determination of the  $C_t$  value, a threshold should be applied to exclude background noise and avoid false-positive signals.

### Thresholds: RGQ

Before determining the  $C_t$ -value with the Rotor-Gene software, check whether the baseline is positioned correctly and adjust if necessary by using the slope correct function. The  $C_t$ -value is determined by using the threshold function. The recommended thresholds for amplification curves are listed in table 7. The threshold locations are based on the maximal fluorescence intensity, but can differ between samples and different Rotor-Gene Q instruments. An example of an amplification curve obtained with the Rotor-Gene Q is illustrated in figure 2.

**Table 7. Thresholds Rotor-Gene Q amplification curves**

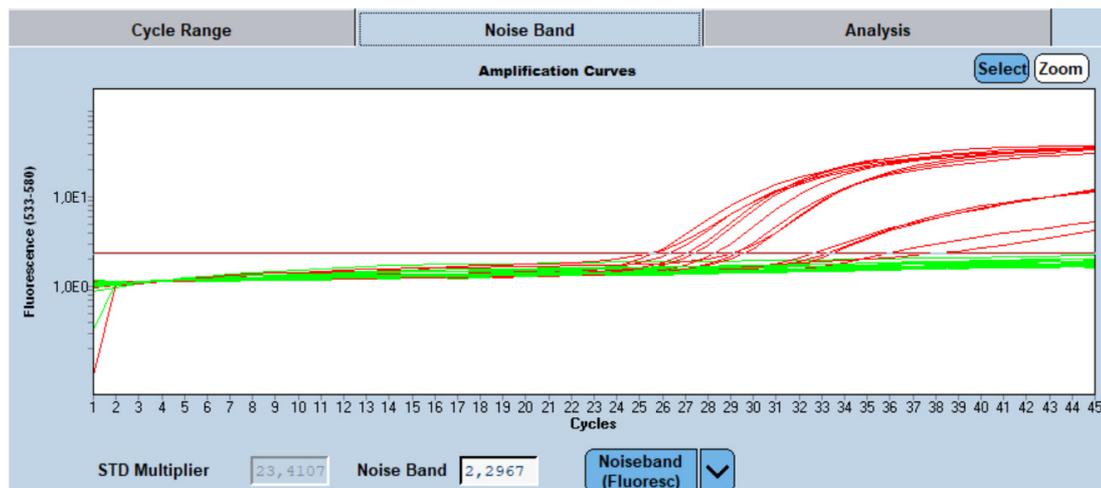
Max FI (norm. fluorescence)	Threshold location
< 0.2	0.025
0.2-0.6	0.05
0.6-1.0	0.1
>1.0	0.15



**Figure 2. Amplification curve.** Example of an amplification curve on the RGQ, obtained with the Mucorales probe for *Rhizopus oryzae* in the yellow detection channel.

### Thresholds: LC480 II

To determine the  $C_t$ -values with the LC480 II software, we recommend to use the Fit points method. This method is more reliable compared to the 2<sup>nd</sup>-derivative method for determination of the  $C_t$ -value for samples with low amounts of Mucorales DNA. This requires manual positioning of the threshold in the noise band section. Make sure that the threshold is positioned slightly higher than the background signals. An example is shown in figure 3. In addition, always check if a sigmoidal amplification curve is present and compare it to the positive control to confirm it's true positivity.



**Figure 3. Threshold positioning in noise band section of Fit points analysis.** As a result of the threshold positioning, the red curves are indicated as positive by the software and will display a  $C_t$ -value, the green curves are indicated as negative by the software.

### Thresholds: CFX96

To determine the  $C_t$ -values with the CFX96 software, we recommend to use the  $C_q$  determination mode and select regression. This function automatically determines the  $C_q$ -value. The option single threshold can be selected for selection of a manual threshold, but is not recommended as this is not standardized.

Thresholds: QS5

The  $C_t$ -values are automatically determined with the Quantstudio design & analysis software using an automated threshold function. It is recommended to use a PCR run template prepared by PathoNostics.

Thresholds: Mic

To automatically determine the  $C_t$ -values on the Mic qPCR, the auto set threshold function can be selected. It is recommended to use the PCR run template prepared by PathoNostics.

## 14 Interpretation of results

Data interpretation of the MucorGenius® PCR is shown in table 8. If no fluorescence signal in any detection channel is observed when using the MucorGenius® real-time PCR, the DNA is degraded, the sample is inhibited, or a manual error has occurred.

**Table 8. Interpretation of signals with the MucorGenius®**

MucorGenius® amplification signals			
LC480	533-580	618-660	Result
RGQ	yellow	Red	
CFX	HEX	Cy5	
MIC	yellow	Red	
QS5	VIC	Cy5	
-	+	+	<i>Mucorales</i> spp
-	-	+	No <i>Mucorales</i> spp
-	+	-	IC outcompeted by high <i>Mucorales</i> spp. load; result is still valid
-	-	-	Invalid

 $C_t$  cut-off value

No fixed  $C_t$  cut-off value can be advised due to the limited number of positive samples included in the clinical studies as a result of the rarity of the disease (no statistical justification). In general,  $<C_t 40$  should always be considered as a positive result while  $>C_t 40$  are considered as a negative result.

Controls

To check for a correct DNA extraction (IC) and PCR procedure (IC and PC), the  $C_t$ -values for all controls included in the MucorGenius® PCR must be within the acceptable range (table 9). The  $C_t$ -value for the IC is strongly dependent on the sample matrix and the DNA-extraction procedure. However, a positive signal for the IC must be detected in *Mucorales* spp. negative samples.

**Table 9 C<sub>t</sub> value ranges for controls.** C<sub>t</sub>-values were determined by using the described threshold settings for all real-time cyclers.

Control	Target	Channel	Ct-values
MucorGenius® PC	<i>Mucorales</i> spp.	Yellow/533-580/HEX/VIC	25.0-29.0
IC*	M13	Red/618-660/Cy5	30.0-36.0

\* A strong positive *Mucorales* spp. sample might outcompete the IC and result in higher C<sub>t</sub> values than listed.

## 15 Troubleshooting

This troubleshooting section may be helpful in solving any problems that may arise (table 10).

**Table 10. Troubleshooting**

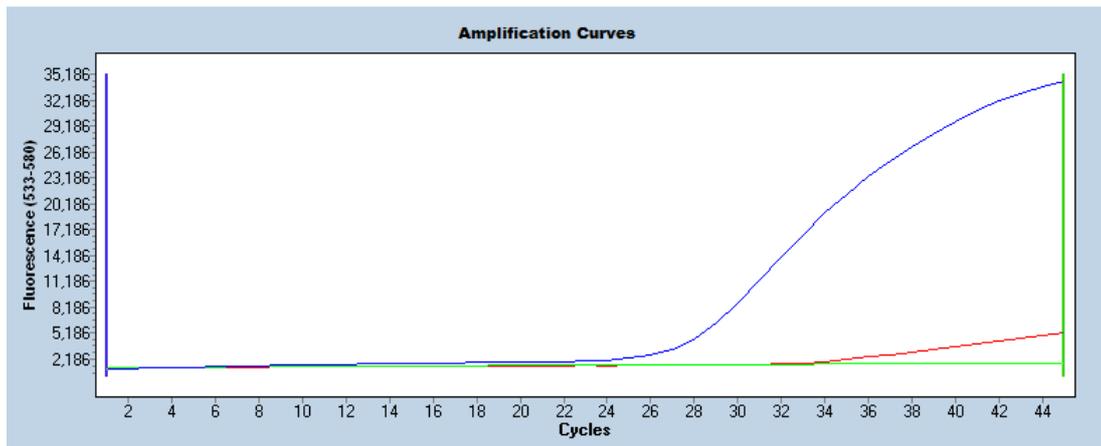
Problem	Possible cause	Recommendations
Positive controls remain negative	<p>One of the components was not added</p> <p>The control was not stored properly</p> <p>Positive control was added to the wrong reaction tube</p> <p>Incorrect PCR profile / programming</p>	<p>Ensure that all components have been added</p> <p>Check storage conditions and the expiry date of the kit (indicated on the box label)</p> <p>Ensure that positive control was added to the correct reaction tube.</p> <p>Check programming of real-time cycler</p> <p>Check your work steps / pipetting scheme and check calibration of real-time PCR machine and pipettes.</p>
IC remains negative in <i>Mucorales</i> spp- negative samples	<p>Nucleic acid degradation / inhibition</p> <p>Incorrect PCR mixture</p> <p>PCR conditions do not comply with the protocol</p> <p>Incorrect elution volume / insufficient IC added</p> <p>DNA extraction procedure is used which is not recommended and results in less efficient IC extraction yield</p>	<p>Repeat the extraction according to the protocol and ensure that all instruments are properly decontaminated</p> <p>Check if sufficient PCR mix, Taq polymerase and sample are added</p> <p>Check PCR conditions and repeat the PCR with correct settings if necessary</p> <p>In case the elution is performed in 50 µl, check if 5 µl IC is added to the starting material.</p> <p>Contact PathoNostics for experience with the particular DNA extraction platform. It can be recommended to repeat the extraction with 10 µl IC material, depending on the extraction procedure.</p>
Negative Control generates a fluorescence signal	Carry over / contamination	Repeat the entire experiment with fresh reagents, handle samples, kit components and consumables as prescribed and spin down reagents before pipetting

		<p>If the problem still occurs, repeat also the DNA-extraction</p> <p>Make sure you performed all steps in a laminar air flow safety cabinet II in order to prevent contamination.</p> <p>Add positive controls strictly at the last step.</p> <p>Make sure that work spaces and instruments are decontaminated regularly.</p>
Very weak fluorescence signals also for controls	<p>Incorrect instrument settings</p> <p>Incorrect real-time PCR mix</p>	<p>Check channel settings</p> <p>Check if the PCR mixture is prepared according to the protocol</p> <p>Check expiry date and storage conditions</p>

**Problems in data-analysis that may arise**

Limited sigmoidal curves

Samples with low amounts of Mucorales DNA (e.g. close to the detection limit) can result in less sigmoidal amplification curves (figure 4). This is a result of a reduced amplification efficiency. Always compare these signals to the positive and negative control signals. When the curve shows an increase compared to the background, the sample must be considered positive. Use the described threshold settings for the specific real-time PCR cyclers to accurately identify the signal. Usually these signals are observed at Ct 33 or higher.



**Figure 4. Limited sigmoidal signals for samples with low amounts of Mucorales DNA.** Use the correct threshold settings and always compare the Mucorales signal of the sample (red) to the positive control (blue) and negative control (green).

## 16 Notice to the purchaser

The MucorGenius® product is manufactured by PathoNostics B.V. in Maastricht, The Netherlands within quality systems accredited to ISO 13485:2016. This product is sold to use by the end-user only and may only be re-sold, distributed or re-packaged with approval of PathoNostics and only by licensed distributors.

Reagents of different MucorGenius® lots should not be combined.

If a MucorGenius® kit is received in a damaged packaging, please contact PathoNostics or your local PathoNostics distributor.

Other language versions of these Instructions for Use can be requested from PathoNostics or your local PathoNostics distributor.

### **Disclaimer**

The results obtained from these or any other diagnostic panels should be used and interpreted only in the context of the overall clinical picture. PathoNostics BV cannot accept responsibility for any clinical decisions that are made. PathoNostics BV does not represent this guide as a comprehensive summary of all possible outcomes from using the MucorGenius® real-time PCR kits. This guide is intended for use solely as an aid to memory. It is not for use in any clinical interpretation of the results of the assay. Laboratories must interpret and report the results of the assay in accordance with their own locally developed procedures.

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## 17 Performance characteristics

### 17.1 Limit of detection (LoD)

The LoD or analytical sensitivity was determined for several Mucorales species using dilution series of genomic DNA. The final LoD was confirmed by testing 20 replicates with a positivity rate of  $\geq 95\%$ . Replicates were spiked with the internal control. Quantification of the targets was performed with an in-house droplet digital PCR. An overview of the LoD values for all targets are shown in table 11.

**Table 11. LoD values of MucorGenius®**

Target	Strain	Tested conc (c/ $\mu$ l)	LC480	RGQ	CFX96	Mic	QS5
			Ct-value				
<i>R. oryzae</i>	IHEM06016	2	32-35	33-35	31-32	32-33	34-40
<i>R. microsporus</i>	IHEM22234	2	33-34	34-43	32-33	34-36	31-38
<i>R. miehei</i>	IHEM26897	1	33-34	34-39	33-35	33-37	34-40
<i>R. pusillus</i>	IHEM21236	2	33-34	34-37	32-33	33-34	31-36
<i>C. bertholletiae</i>	In-house*	1	32-33	32-38	34-40	32-35	31-38
<i>L. corymbifera</i>	In-house*	1	32-33	33-37	33-41	32-35	28-38
<i>M. hiemalis</i>	IHEM27256	2	32-33	32-37	31-32	32-33	33-40

\*Confirmed by 18S sequencing

### 17.2 Analytical specificity

The analytical specificity of the MucorGenius® real-time PCR was determined by testing DNA of various relevant respiratory pathogens including various fungal strains and bacteria. All DNA samples tested are listed in table 12. In addition, primer and probes were checked for possible homologies to all sequences published in GenBank (NCBI) by sequence comparison analysis to ensure specificity of the MucorGenius® assay.

**Table 12. Testing of the specificity of relevant strains. + = positive, - = negative**

Pathogen	Reference	Mucorales spp
<i>R. oryzae</i>	IHEM 06016	+
<i>C. bertholletiae</i>	In-house*	+
<i>L. corymbifera</i>	In-house*	+
<i>M. hiemalis</i>	IHEM 27256	+
<i>R. pusillus</i>	IHEM 21236	+
<i>R. miehei</i>	IHEM 26897	+
<i>R. microsporus</i>	IHEM 22234	+
<i>S. prolificans</i>	IHEM 21157	-
<i>P. marneffeii</i>	ATCC 18224D	-
<i>P. chrysogenum</i>	IHEM 18723	-

<i>F. solani</i>	IHEM 25864	-
<i>P. jirovecii</i>	In-house*	-
<i>S. cerevisiae</i>	IHEM 25104	-
<i>C. neoformans</i>	IHEM 04165	-
<i>A. fumigatus</i>	ATCC MYA4609	-
<i>A. flavus</i>	In-house*	-
<i>A. terreus</i>	In-house*	-
<i>A. niger</i>	In-house*	-
<i>C. albicans</i>	In-house*	-
<i>C. parapsilosis</i>	In-house*	-
<i>C. krusei</i>	In-house*	-
<i>C. glabrata</i>	In-house*	-
<i>M. tuberculosis</i>	BCCM000092	-
<i>M. pneumoniae</i>	ATCC 29085	-
<i>L. pneumophila</i>	ATCC 33152	-
<i>K. pneumoniae</i>	In-house*	-
<i>S. pneumoniae</i>	ATCC 700669D-5	-
<i>M. catarrhalis</i>	In-house*	-
<i>H. influenzae</i>	ATCC 51907D	-
<i>B. pertussis</i>	ATCC BAA-589	-
<i>E. coli</i>	ATCC 700973	-
<i>P. auruginosa</i>	In-house*	-
<i>S. aureus</i>	ATCC BAA-1556D-5	-
Human genomic DNA	Promega	-

\*Confirmed by sequencing

### 17.3 Robustness and interfering substances

The verification of the robustness allows the determination of the total failure rate of the MucorGenius® PCR by monitoring the presence of the internal control. The robustness was verified by testing 20 BAL samples negative for Mucorales species. For this purpose, two different DNA-extraction methods were used for: The NucliSENS® EasyMag® (bioMérieux) and QIAamp MinElute Virus Spin kit (Qiagen; nr 57704). For both methods, 200 µl BAL and 50 µl elution volume was used and all samples were analysed with the MucorGenius® PCR. The IC was detected in all samples at expected Ct-values (Ct 30-32). As a result, the robustness for the MucorGenius® PCR assay is 100%.

The MucorGenius® PCR was tested for the interference by high loads of human DNA (100 ng/µl). Detection of Mucorales was comparable to Mucorales samples without the presence of human DNA and therefore detection of the MucorGenius® PCR assay was not compromised by high loads of human DNA

### 17.4 Reproducibility and accuracy

To determine the reproducibility or accuracy, three different MucorGenius® lots were tested on several targets and on two different real-time PCR instruments (LC480 II and RGQ). The

arithmetic mean, standard deviation (stdev) and coefficient of variation (CV) were calculated. The results are listed in table 13 (LC480 II) and 14 (RGQ).

**Table 13. Reproducibility of the MucorGenius® on the LC480.**

Sample nr	LC480 II					
	Lot 1	Lot 2	Lot 3	Mean	stdev	CV (%)
<i>R. oryzae</i>	32.1	32.2	32.1	32.1	0.07	0.22
<i>C. bertholletiae</i>	30.5	30.7	30.5	30.6	0.13	0.42
<i>R. pusillus</i>	32.0	32.0	32.3	32.1	0.17	0.53
Positive control	27.2	27.2	27.3	27.2	0.06	0.22
Internal control	32.3	32.7	32.7	32.6	0.23	0.71

**Table 14. Reproducibility of the MucorGenius® on the RGQ.**

Sample nr	RGQ					
	Lot 1	Lot 2	Lot 3	Mean	stdev	CV (%)
<i>R. oryzae</i>	34.1	34	35.3	34.5	0.73	2.12
<i>C. bertholletiae</i>	30.3	29	29.4	29.6	0.67	2.26
<i>R. pusillus</i>	33.2	32.6	32.1	32.6	0.52	1.60
Positive control	25.7	25.3	25.8	25.6	0.23	0.90
Internal control	29.7	29.8	29.7	29.7	0.17	0.57

### 17.5 FPCR

The performance of the MucorGenius® real-time PCR kit was determined by using the an External Quality Assessment (EQA) program from the European Fungal PCR Initiative (FPCR). The serum panel contained 7 samples and DNA was extracted with the NucliSENS® EasyMag® (bioMérieux) followed by elution in 50 µl. The results with the MucorGenius® PCR kit is shown in table 15 (100% score) and was obtained by detection on the LC480 II. The individual test report can be requested at PathoNostics or your local distributor.

**Table 15. Results MucorGenius® on the FPCR serum panel.**

Sample nr	Inoculated DNA (Equivalent genomes/ml)	Results		
		Mucorales detection	Genus/species identification	Ct
S1	No	Negative	Negative	
S2	<i>R. pusillus</i> (10)	Positive	Mucorales	35.7
S3	<i>L. corymbifera</i> (100)	Positive	Mucorales	30.6
S4	<i>L. corymbifera</i> (1)	Positive	Mucorales	35
S5	<i>R. pusillus</i> (100)	Positive	Mucorales	32.5
S6	<i>L. corymbifera</i> (10)	Positive	Mucorales	33.5
S7	<i>R. pusillus</i> (1)	Positive	Mucorales	36.2

## 18 Symbols

	<N>	Contains reagents sufficient for <N> reactions
		Use by
		In vitro diagnostic medical device
		Catalog number
		Lot number
		Temperature limitation
		Manufacturer
		Consult instructions for use
		Caution
		Keep away from light

## 19 Contact

For technical assistance and more information, please contact us by sending an email to [info@pathonostics.com](mailto:info@pathonostics.com) or call +31-43-3030423. For technical assistance please refer to the catalog number PN-700.

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## 20 Purchase order information

To place an order, contact the customer service department at [info@pathonostics.com](mailto:info@pathonostics.com) or fill in an on-line order form at [www.pathonostics.eu/contact](http://www.pathonostics.eu/contact).

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