

Quantiplus® Mucormycosis PCR Kit

(Real-time Qualitative PCR Kit)



QL-MUCO-25 : 25 rxns
 QL-MUCO-50 : 50 rxns



PI/QLMUCO-00

Introduction & Product Description

Mucorales have been increasingly reported as causes of invasive fungal infections in immune compromised subjects, particularly in patients with haematological malignancies, uncontrolled diabetes mellitus or those undergoing dialysis and off lately subjects recovering from covid19 infection. Mucorales are now also being reported in Aspergillus-positive patients who are not responding to first line treatments. Histology and culture are still the most important diagnostic approaches for mucormycosis because of the lack of molecular diagnostic methods available, and β -d-Glucan detection is not useful due to the extremely low content of the biomarker in the Mucorales order. Timely diagnosis of invasive mucormycosis is essential due to the rapid progression of the disease, and because signs and symptoms of the infection are consistent with other invasive fungal infections. Mucorales lacks diagnostics and it is suspected that prevalence is higher than reported, therefore the use of PCR could improve the sensitivity of Mucorales detection and influence appropriate treatment decisions more rapidly. The Quantiplus® Mucormycosis PCR Kit is designed to detect DNA of Mucorales species including Rhizopus spp, Mucor spp, Lichtheimia spp, Cunninghamella spp and Rhizomucor spp.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL-MUCO-25)	50 rxns (QL-MUCO-50)
Green	D Fast Core qPCR Mix (2X)	Amplification reagent	1 x 325 μ L	1 x 750 μ L
Amber	Muco PPM	Muco, internal control primer probe mix	1 x 50 μ L	1 x 100 μ L
Natural	Huwel IC-B Mix	Internal Control	1 X 300 μ L	1 X 600 μ L
Red	Muco PC	Muco Positive Control	1 x 50 μ L	1 x 100 μ L
White	Huwel PW	Purified water	1 x 500 μ L	1 x 500 μ L

Sample Type

Plasma (K2EDTA-Blood), BAL Fluid, CSF, Biopsy. Heparinized Blood must not be used as they inhibit the PCR reaction

Assay Procedure

DNA Extraction

- 1- Huwel Fungal DNA extraction kit (HL-FDX-100)

qPCR Protocol

Components	Volume per reaction (μ L) (For final vol. of 25 μ L)
D Fast Core qPCR Mix (2X)	13.0
Muco PPM	2.0
Huwel IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ Muco PC/ Huwel PW	5.0

Cycling conditions

Steps	No. of cycles	Temperature (°C)	Time
Enzyme Activation	1	50	2min
1 (Initial denaturation)	1	95	15 min.
2 (PCR cycling-1)	40	95	15 sec.
		*60	40 sec.
* Plate Read/Data Acquisition in FAM and Yakima Yellow/VIC channel			

- Set the reaction volume as 25 µL

Data Analysis

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to $R_n/Cycle$ amplification plot.

Result and Interpretation

S.No	FAM (MUCO)	Yakima Yellow / VIC (IC)	← Fluorophore	Conclusion
			Interpretation ↓	
1	√	√	Muco DNA detected	Proceed for further Analysis
2	√	-	Muco DNA detected	
3	-	√	Muco DNA Not detected	
4	-	-	Possible inhibition of PCR	Make sure reaction setup and PCR conditions are followed correctly Recheck quality and quantity of DNA

Assay Characteristics

Analytical Sensitivity of qPCR assay is **50 copies/mL**

Specificity

The analytical specificity of the Quantiplus® Mucormycosis PCR Kit was assured by primer design and stringent PCR conditions. Blast search was done to avoid any homology on primers with other organisms.



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