Quantiplus® Panfungal, Aspergillus and Candida RT PCR Kit
(Real-Time Qualitative PCR Kit)

QL-PAC-25 : 25 rxns
QL-PAC-50 : 50 rxns

RUO

PI/QLPAC-00

Introduction & Product Description

Invasive fungal infections (IFIs) remain a major cause of morbidity and mortality in immunocompromised patients, and diagnosis continues to be problematic. Laboratory diagnosis of IFIs is based on classical methods such as fungus isolation in culture and histopathological examination; however, these methods present several limitations. Fungal cultures are frequently slow growing, and such assays are low in sensitivity as well as in specificity because fungi are habitual laboratory contaminants and part of the saprophytic human flora.

Quantiplus® Panfungal, Aspergillus and Candida RT PCR Kit is a nucleic acid amplification test for the qualitative detection of *Fungal species* in human clinical specimens. The kit contains all the reagents necessary for performing qualitative Panfungal Realtime PCR. Pathogen detection by Real time Polymerase chain reaction (PCR) is based on the amplification of ITS (internal transcribed spacer) region of rDNA from the pathogen genome. The assay principle is based on TaqMan based Realtime PCR which allows higher specificity and sensitivity.

Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QL-PAC-25)	50 rxns (QL-PAC-50)
Amber	RM-A	Panfungal Amplification Mix	1 x 375 µL	1 x 750 µL
Amber	RM-B	Aspergillus and Candida Amplification Mix	1 x 375 µL	1 x 750 µL
Natural	Huwel IC-B Mix	Internal Control	1 X 300 µL	1 X 600 µL
Red	Huwel Fungal PC	Panfungal Positive Control	1 x 50 µL	1 x 100 µL
Red	Huwel Fac PC	Aspergillus and Candida Positive Control	1 x 50 µL	1 x 100 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL

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-50/100)

qPCR Protocol

Components	Volume per reaction (µL) (For final vol. of 26 µL)
RM-A	15.0
Huwel IC-B Mix (If not added at extraction step)	1.0
Extracted DNA/ Huwel Fungal PC / Huwel PW	10.0

Note: *If the Panfungal result shows positive for clinical specimen, proceed for Genus specific Fungal PCR assay*

Reaction setup for Genus specific PCR assay

Components	Volume per reaction (µL) (For final vol. of 25 µL)
RM-B	15.0
Extracted DNA/ Huwel Fac PC / Huwel PW	10.0

Cycling conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	15 min.
2 (Cycling)	35	95	15 sec.
		60*	60 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 (Panfungal)	HEX /VIC (Internal Control)	Fluorophore / Ct value	Interpretation	Conclusion
1	√	√	< 32Ct	Panfungal DNA detected	Proceed for further Analysis
2	√	-	< 32Ct	Panfungal DNA detected	
3	-	√	No Amplification or > 32 Ct	Panfungal DNA Not detected	
4	-	-	-	Possible inhibition of PCR	Make sure reaction setup and PCR conditions are followed correctly Recheck quality and quantity of DNA

Interpret the values for unknown Genus specific samples as described in the table-2

S.No	FAM (Candida)	HEX /VIC (Aspergillus)	Fluorophore / Ct value	Interpretation	Conclusion
1	√	-	< 32Ct	Candida DNA detected	Proceed for further Analysis
2	-	√	< 32Ct	Aspergillus DNA detected	
3	-	-	No Amplification or > 32Ct	Aspergillus and Candida DNA not detected	

Assay Characteristics

The analytical sensitivity of Quantiplus® Panfungal, Aspergillus and Candida RT PCR Kit is **100 copies** per reaction

Specificity

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