

PathoSEEK® Pan Pythium Detection Assay

Method Developer Validation

Introduction

The oligos for the Pan Pythium assay were designed to amplify 5 different species reported to be found on cannabis ([Table 1](#); Lung et al 2021)¹:

- *Pythium aphanidermatum*
- *Pythium catenulatum*
- *Pythium dissotocum*
- *Pythium myriotylum*
- *Pythium ultimum*

Method Validation

Our team collaborated with plant pathologist Zamir K. Punja, PhD from the Department of Biological Sciences, Simon Fraser University. We received root samples from cannabis plants confirmed to be infected with *Pythium*. DNA was extracted from the samples, using the Medicinal Genomics Quick Lysis Solution and tested using the PathoSEEK® Pan Pythium Detection Assay. Table 1 below shows the results from those tests. Cq values under 35 indicate a positive result. All samples tested positive as expected.

Table 1: Roots tested for *Pythium*

Sample	Target (FAM)	Cq (FAM)	Target (HEX)	Cq (HEX)
PK - Roots from veg plant	Pythium	22.00	ICC	23.70
JFG - Roots from veg plant	Pythium	30.80	ICC	24.01
PG - Roots from veg plant	Pythium	23.82	ICC	23.78
CSDS - Roots from veg plant	Pythium	30.07	ICC	23.92
DB - Roots from veg plant	Pythium	30.14	ICC	23.38
BF - Roots from veg plant	Pythium	26.25	ICC	23.60
PK - Roots from flowering plant	Pythium	29.81	ICC	24.64
JGF - Roots from flowering plant	Pythium	30.92	ICC	23.64
PG - Roots from flowering plant	Pythium	25.68	ICC	24.50

¹ <https://www.tandfonline.com/doi/full/10.1080/07060661.2021.1954695>

CDS - Roots from flowering plant	Pythium	25.71	ICC	24.73
DB - Roots from flowering plant	Pythium	24.01	ICC	23.90
BF - Roots from flowering plant	Pythium	28.86	ICC	24.19
El Gordo - Roots from a mother plant	Pythium	26.21	ICC	25.59
PD - Roots from a mother plant	Pythium	24.38	ICC	24.75
HM #23 - Roots from mother plant organic	Pythium	30.46	ICC	28.92
HM #17 - Roots from mother plant organic	Pythium	26.86	ICC	25.83
SLH C3 roots from flowering plant organic	Pythium	32.53	ICC	32.77
SLH C3 roots from flowering plant organic	Pythium	32.01	ICC	30.61
Pos Control diluted 1:1M	Pythium	19.22	ICC	N/A
NTC	Pythium	ND	ICC	ND

Limits of Detection

The Limit of Detection (LOD) is a measure of the qPCR assay's sensitivity. The LOD was tested by doing a 10-fold serial dilution using the *Pythium* positive control in a cannabis matrix. The approximate copy number was determined using the provided OD260 and amplicon length. This experiment was performed on two different platforms. The first was the Biorad CFX96 qPCR Instrument. The second was the Biomolecular Systems Mic qPCR Instrument along with the Myra Liquid Handling System.

The study, shown below in Table 2, indicates that the PathoSEEK Pan Pythium Detection Assay can reliably detect down to 8 copies. Please note that Cq values greater than 35 are determined inconclusive.

Table 2: *Pythium* LOD Spike-in with matrix

Sample	Amount of Molecules	Matrix	Cq Fam	Cq Hex
Pythium gBlock Control	7,940,000	Yes	14.33	27.86
Pythium gBlock Control	7,940,000	Yes	14.42	28.11
Pythium gBlock Control	7,940,000	Yes	14.22	26.79
Pythium gBlock Control	7,940,000	Yes	14.50	28.08
Pythium gBlock Control	7,940,000	Yes	14.39	27.77
Pythium gBlock Control	794,000	Yes	17.38	27.67
Pythium gBlock Control	794,000	Yes	17.37	27.58

Pythium gBlock Control	794,000	Yes	17.19	26.88
Pythium gBlock Control	794,000	Yes	17.28	26.80
Pythium gBlock Control	794,000	Yes	17.04	25.76
Pythium gBlock Control	79,400	Yes	20.48	27.50
Pythium gBlock Control	79,400	Yes	20.41	27.36
Pythium gBlock Control	79,400	Yes	20.45	27.25
Pythium gBlock Control	79,400	Yes	20.19	25.79
Pythium gBlock Control	79,400	Yes	20.68	27.35
Pythium gBlock Control	7,940	Yes	23.95	27.47
Pythium gBlock Control	7,940	Yes	23.70	27.26
Pythium gBlock Control	7,940	Yes	23.72	27.19
Pythium gBlock Control	7,940	Yes	23.56	26.19
Pythium gBlock Control	7,940	Yes	23.25	25.25
Pythium gBlock Control	794	Yes	27.08	27.31
Pythium gBlock Control	794	Yes	27.15	27.35
Pythium gBlock Control	794	Yes	27.06	27.32
Pythium gBlock Control	794	Yes	27.03	26.33
Pythium gBlock Control	794	Yes	26.91	25.99
Pythium gBlock Control	79	Yes	30.32	27.41
Pythium gBlock Control	79	Yes	30.24	27.30
Pythium gBlock Control	79	Yes	30.38	27.31
Pythium gBlock Control	79	Yes	30.09	26.34
Pythium gBlock Control	79	Yes	29.93	25.55
Pythium gBlock Control	8	Yes	33.37	27.52
Pythium gBlock Control	8	Yes	33.56	27.51
Pythium gBlock Control	8	Yes	33.41	27.42
Pythium gBlock Control	8	Yes	33.33	26.03
Pythium gBlock Control	8	Yes	33.78	28.03
Pythium gBlock Control	1	Yes	37.42	27.63
Pythium gBlock Control	1	Yes	37.57	27.61
Pythium gBlock Control	1	Yes	36.02	27.57
Pythium gBlock Control	1	Yes	37.52	27.49
Pythium gBlock Control	1	Yes	37.06	27.44

	NTC	Yes	ND	ND
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Inclusivity and Exclusivity Study

Table 3 below shows an inclusivity study performed using an *in silico* analysis of the PathoSEEK Pan Pythium Detection Assay primers against NCBI genomes. The table also includes results from testing two *Pythium* species provided by Dr. Punja using the PathoSEEK Pan Pythium Detection Assay. Table 4 shows an exclusivity study performed on 50 live organisms acquired from ATCC (American Type Culture Collection).

Table 3: Pan Pythium Inclusivity

Organism	Source	Assay	NCBI taxid	Max Alignment Score Percent	qPCR
<i>Pythium aphanidermatum</i>	N/A	Pan Pythium	65070	100.00%	N/A
<i>Pythium catenulatum</i>	N/A	Pan Pythium	83917	100.00%	N/A
<i>Pythium dissotocum</i>	Simon Fraser University	Pan Pythium	82936	100.00%	14.27
<i>Pythium myriotylum</i>	Simon Fraser University	Pan Pythium	82941	100.00%	14.82
<i>Pythium ultimum</i>	N/A	Pan Pythium	2052682	100.00%	N/A

Table 4: Pan Pythium Exclusivity

Organism	Source (ATCC Catalog Number)	Assay Target	Ct (FAM)
<i>Alternaria alternata</i>	6663	Pan Pythium	ND
<i>Acinetobacter baumannii</i>	19606	Pan Pythium	ND
<i>Aspergillus alabamensis</i>	MYA-3633	Pan Pythium	ND
<i>Aspergillus caesillus</i>	42693	Pan Pythium	ND
<i>Aspergillus carneus</i>	13549	Pan Pythium	ND
<i>Aspergillus deflectus</i>	62502	Pan Pythium	ND
<i>Aspergillus fijinsis Varga et al</i>	20611	Pan Pythium	ND
<i>Aspergillus fischeri</i>	66641	Pan Pythium	ND
<i>Aspergillus flavus</i>	MYA-3631	Pan Pythium	ND
<i>Aspergillus flavus</i>	MYA-200026	Pan Pythium	ND
<i>Aspergillus fumigatus</i>	13073	Pan Pythium	ND

<i>Aspergillus japonicus</i>	16873	Pan Pythium	ND
<i>Aspergillus nidulans</i>	38163	Pan Pythium	ND
<i>Aspergillus niger</i>	13496	Pan Pythium	ND
<i>Aspergillus oryzae</i>	10124	Pan Pythium	ND
<i>Aspergillus parasiticus</i>	56775	Pan Pythium	ND
<i>Aspergillus pseudo terreus Peterson et al</i>	10020	Pan Pythium	ND
<i>Aspergillus terreus</i>	20542	Pan Pythium	ND
<i>Aspergillus tubingensis</i>	1004	Pan Pythium	ND
<i>Aspergillus tubingensis</i>	MYA 4996	Pan Pythium	ND
<i>Aspergillus ustus</i>	1041	Pan Pythium	ND
<i>Aspergillus versicolor</i>	11730	Pan Pythium	ND
<i>Candida albicans</i>	10231	Pan Pythium	ND
<i>Cryptococcus neoformans</i>	208821	Pan Pythium	ND
<i>Fusarium oxysporum</i>	Punja	Pan Pythium	ND
<i>Fusarium solani</i>	52628	Pan Pythium	ND
<i>Mucor hiemalis</i>	28935	Pan Pythium	ND
<i>Mucor luteus</i>	28932	Pan Pythium	ND
<i>Penicillium chrysogenum</i>	18476	Pan Pythium	ND
<i>Penicillium rubens</i>	11709	Pan Pythium	ND
<i>Penicillium marneffeii</i>	18224	Pan Pythium	ND
<i>Yarrowia lipolytica</i>	20390	Pan Pythium	ND
<i>Vibrio cholerae</i>	39415D-5	Pan Pythium	ND
<i>E. coli (STEC)</i>	BAA-2440D	Pan Pythium	ND
<i>Salmonella heutenae</i>	1580	Pan Pythium	ND
<i>Salmonella bongori</i>	43975	Pan Pythium	ND
<i>E.coli</i>	2326	Pan Pythium	ND
<i>Enterobacter aerogenes</i>	13040	Pan Pythium	ND
<i>Listeria seeligeri</i>	35967D-5	Pan Pythium	ND
<i>Listeria welshimeri</i>	35897D-5	Pan Pythium	ND
<i>Listeria monocytogenes</i>	7647	Pan Pythium	ND
<i>Salmonella</i>	700720	Pan Pythium	ND
<i>Salmonella indica</i>	1578	Pan Pythium	ND

<i>Staph aureus</i>	12600	Pan Pythium	ND
<i>Yersinia pestis</i>	BAA-1511D-5	Pan Pythium	ND
<i>Klebsiella pneumoniae</i>	BAA-2146	Pan Pythium	ND
<i>Shigella flexneri</i>	29903D-5	Pan Pythium	ND
<i>Pseudomonas aeruginosa</i>	35554	Pan Pythium	ND
<i>Pseudomonas aeruginosa</i>	13525	Pan Pythium	ND
<i>Lactobacillus</i>	10075	Pan Pythium	ND

REVISION HISTORY

Version	Date	Description
v1	July 2024	Updated <i>Pythium</i> specific validation document

DISCLAIMER

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