

## **PathoSEEK® Pan Pythium and Pan Fusarium Detection Assays**

### **Method Developer Validation**

## **Introduction**

The oligos for the Pan Fusarium assay were designed to amplify 10 different Fusarium species reported to be found on cannabis ([Table5](#); Punja et al 2023)<sup>1</sup>:

- *Fusarium avenaceum*
- *Fusarium brachygibbosum*
- *Fusarium equiseti*
- *Fusarium falciforme*
- *Fusarium graminearum*
- *Fusarium lichenicola*
- *Fusarium oxysporum*
- *Fusarium proliferatum*
- *Fusarium solani*
- *Fusarium sporotrichioides*

The oligos for the Pan Pythium assay were designed to amplify 5 different species reported to be found on cannabis ([Table1](#); Lung et al 2021)<sup>2</sup>:

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

## **Method Validation**

Our team received multiple root samples from cannabis plants infected with Fusarium and/or Pythium that were submitted by a plant pathologist. DNA was extracted from the samples, using the Medicinal Genomics Quick Lysis Solution and tested using the PathoSEEK® Fusarium Detection Assay and the PathoSEEK Pythium Detection Assay. Table 1 below shows the results from those tests.

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<sup>1</sup> <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1278189/full>

<sup>2</sup> <https://www.tandfonline.com/doi/full/10.1080/07060661.2021.1954695>

**Table 1: Roots tested for *Fusarium* and *Pythium***

Sample	Fusarium Cq (FAM)	ICC Cq (HEX)	Pythium Cq (FAM)	ICC Cq (HEX)
PK - Roots from veg plant	N/A	26.40	22.00	23.70
JFG - Roots from veg plant	N/A	27.54	30.80	24.01
PG - Roots from veg plant	38.13	28.12	23.82	23.78
CDSD - Roots from veg plant	N/A	24.41	30.07	23.92
DB - Roots from veg plant	N/A	26.00	30.14	23.38
BF - Roots from veg plant	N/A	25.01	26.25	23.60
PK - Roots from flowering plant	35.71	26.13	29.81	24.64
JGF - Roots from flowering plant	N/A	27.09	30.92	23.64
PG - Roots from flowering plant	N/A	26.26	25.68	24.50
CDSD - Roots from flowering plant	35.68	26.09	25.71	24.73
DB - Roots from flowering plant	33.16	27.46	24.01	23.90
BF - Roots from flowering plant	31.54	25.05	28.86	24.19
El Gordo - Roots from a mother plant	N/A	28.32	26.21	25.59
PD - Roots from a mother plant	N/A	26.83	24.38	24.75
HM #23 - Roots from mother plant organic	28.96	26.94	30.46	28.92
HM #17 - Roots from mother plant organic	27.61	30.24	26.86	25.83
SLH C3 roots from flowering plant organic	Not Tested	Not Tested	32.53	32.77
SLH C3 roots from flowering plant organic	Not Tested	Not Tested	32.01	30.61
Positive Control	15.61	ND	19.22	ND
NTC	ND	ND	ND	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 *Fusarium* and *Pythium* positive controls in a cannabis matrix. The approximate copy number was determined using the provided OD260 and amplicon length. This experiment was performed on both the Biorad CFX96 qPCR Instrument and the Biomolecular Systems Mic qPCR Instrument along with the Myra Liquid Handling System.

These studies, shown below in Tables 2 and 3, indicate that the PathoSEEK Pan *Fusarium* Detection Assay can reliably detect down to 53 copies, and 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: Fusarium LOD Spike-in with matrix**

Sample	Molecules	Matrix	Cq Fam	Cq Hex
Fusarium gBlock Control	5,325,000	Yes	14.60	26.55
Fusarium gBlock Control	5,325,000	Yes	14.58	26.45
Fusarium gBlock Control	5,325,000	Yes	14.51	26.58
Fusarium gBlock Control	5,325,000	Yes	14.59	26.47
Fusarium gBlock Control	5,325,000	Yes	14.43	26.52
Fusarium gBlock Control	532,500	Yes	17.53	25.43
Fusarium gBlock Control	532,500	Yes	17.73	25.54
Fusarium gBlock Control	532,500	Yes	17.64	25.42
Fusarium gBlock Control	532500	Yes	17.61	25.57
Fusarium gBlock Control	532,500	Yes	17.57	25.52
Fusarium gBlock Control	53250	Yes	20.66	25.16
Fusarium gBlock Control	53250	Yes	20.93	25.26
Fusarium gBlock Control	53250	Yes	20.91	25.19
Fusarium gBlock Control	53250	Yes	20.94	25.21
Fusarium gBlock Control	53250	Yes	20.96	25.29
Fusarium gBlock Control	5325	Yes	24.05	25.15
Fusarium gBlock Control	5,325	Yes	23.99	25.21
Fusarium gBlock Control	5,325	Yes	24.01	25.23
Fusarium gBlock Control	5,325	Yes	24.06	25.19
Fusarium gBlock Control	5,325	Yes	24.15	25.31
Fusarium gBlock Control	533	Yes	27.68	25.19
Fusarium gBlock Control	533	Yes	27.70	25.31
Fusarium gBlock Control	533	Yes	27.87	25.31
Fusarium gBlock Control	533	Yes	27.94	25.32
Fusarium gBlock Control	533	Yes	27.72	25.24
Fusarium gBlock Control	53	Yes	31.95	25.30
Fusarium gBlock Control	53	Yes	32.01	25.25
Fusarium gBlock Control	53	Yes	31.96	25.27
Fusarium gBlock Control	53	Yes	31.88	25.28

Fusarium gBlock Control	53	Yes	32.64	25.29
Fusarium gBlock Control	5	Yes	37.47	25.28
Fusarium gBlock Control	5	Yes	37.35	25.32
Fusarium gBlock Control	5	Yes	39.32	25.32
Fusarium gBlock Control	5	Yes	36.68	25.37
Fusarium gBlock Control	5	Yes	36.61	25.28
	NTC	Yes	ND	ND

**Table 3: 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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## REVISION HISTORY

Version	Date	Description
v1	December 2023	New Product Launch
v2	March 2024	Updated LOD Study Data

## DISCLAIMER

This test was developed, and its performance characteristics determined by Medicinal Genomics Company, for laboratory use. Any deviations from the protocol are not supported by MGC. The results may vary based on laboratory conditions.

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