

PathoSEEK® Pan Fusarium Detection Assay

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 ([Table 5](#); Punja et al 2023)¹:

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

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 *Fusarium*. DNA was extracted from the samples, using the Medicinal Genomics Quick Lysis Solution and tested using the PathoSEEK® Pan Fusarium 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.

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

Table 1: Roots tested for *Fusarium*

Sample	Target (FAM)	Fusarium Cq (FAM)	Target (HEX)	ICC Cq (HEX)
PG - Roots from veg plant	Fusarium	38.13	ICC	28.12
PK - Roots from flowering plant	Fusarium	35.71	ICC	26.13
CDSD - Roots from flowering plant	Fusarium	35.68	ICC	26.09
DB - Roots from flowering plant	Fusarium	33.16	ICC	27.46
BF - Roots from flowering plant	Fusarium	31.54	ICC	25.05
HM #23 - Roots from mother plant organic	Fusarium	28.96	ICC	26.94
HM #17 - Roots from mother plant organic	Fusarium	27.61	ICC	30.24
Positive Control	Fusarium	15.61	ICC	ND
NTC	Fusarium	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 *Fusarium* 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 Table 2, indicate that the PathoSEEK Pan *Fusarium* Detection Assay can reliably detect down to 53 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

Inclusivity and Exclusivity Study

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

Table 3: Fusarium Inclusivity Study

Organism	Source	Assay	NCBI taxid	Max Alignment Score Percent	qPCR
<i>Fusarium avenaceum</i>	N/A	Pan Fusarium	65070	100.00%	N/A
<i>Fusarium brachygibbosum</i>	N/A	Pan Fusarium	83917	100.00%	N/A
<i>Fusarium equiseti</i>	N/A	Pan Fusarium	82936	100.00%	N/A
<i>Fusarium falciforme</i>	N/A	Pan Fusarium	82941	100.00%	N/A
<i>Fusarium graminearum</i>	N/A	Pan Fusarium	2052682	100.00%	N/A
<i>Fusarium lichenicola</i>	N/A	Pan Fusarium	42744	100.00%	N/A
<i>Fusarium oxysporum</i>	ATCC 62506	Pan Fusarium	5507	100.00%	26.72
<i>Fusarium proliferatum</i>	ATCC 76097	Pan Fusarium	948311	100.00%	26.80
<i>Fusarium solani</i>	ATCC 52628	Pan Fusarium	169388	100.00%	29.62
<i>Fusarium sporotrichioides</i>	N/A	Pan Fusarium	5514	100.00%	N/A

Table 4: Fusarium Exclusivity Study

Organism	ATCC Number	Assay	Ct FAM
<i>Alternaria alternata</i>	6663	Pan Fusarium	ND
<i>Acinetobacter baumannii</i>	19606	Pan Fusarium	ND
<i>Aspergillus alabamensis</i>	MYA-3633	Pan Fusarium	ND
<i>Aspergillus caesillus</i>	42693	Pan Fusarium	ND
<i>Aspergillus carneus</i>	13549	Pan Fusarium	ND
<i>Aspergillus deflectus</i>	62502	Pan Fusarium	ND
<i>Aspergillus fjinsis Varga et al</i>	20611	Pan Fusarium	ND
<i>Aspergillus fischeri</i>	66641	Pan Fusarium	ND
<i>Aspergillus flavus</i>	MYA-3631	Pan Fusarium	34.81*
<i>Aspergillus flavus</i>	MYA-200026	Pan Fusarium	28.99*
<i>Aspergillus fumigatus</i>	13073	Pan Fusarium	ND
<i>Aspergillus japonicus</i>	16873	Pan Fusarium	ND
<i>Aspergillus nidulans</i>	38163	Pan Fusarium	ND
<i>Aspergillus niger</i>	13496	Pan Fusarium	ND
<i>Aspergillus oryzae</i>	10124	Pan Fusarium	29.29*
<i>Aspergillus parasiticus</i>	56775	Pan Fusarium	ND
<i>Aspergillus pseudo terreus Peterson et al</i>	10020	Pan Fusarium	ND
<i>Aspergillus terreus</i>	20542	Pan Fusarium	ND
<i>Aspergillus tubingensis</i>	1004	Pan Fusarium	ND
<i>Aspergillus tubingensis</i>	MYA 4996	Pan Fusarium	ND
<i>Aspergillus ustus</i>	1041	Pan Fusarium	ND
<i>Aspergillus versicolor</i>	11730	Pan Fusarium	ND
<i>Candida albicans</i>	10231	Pan Fusarium	ND
<i>Cryptococcus neoformans</i>	208821	Pan Fusarium	ND
<i>Mucor hiemalis</i>	28935	Pan Fusarium	ND
<i>Mucor luteus</i>	28932	Pan Fusarium	ND
<i>Penicillium chrysogenum</i>	18476	Pan Fusarium	ND
<i>Penicillium rubens</i>	11709	Pan Fusarium	ND
<i>Penicillium marneffeii</i>	18224	Pan Fusarium	ND
<i>Yarrowia lipolytica</i>	20390	Pan Fusarium	ND
<i>Vibrio cholerae</i>	39415D-5	Pan Fusarium	ND

<i>E. coli (STEC)</i>	BAA-2440D	Pan Fusarium	ND
<i>Salmonella heutenae</i>	1580	Pan Fusarium	ND
<i>Salmonella bongori</i>	43975	Pan Fusarium	ND
<i>E.coli</i>	2326	Pan Fusarium	ND
<i>Enterobacter aerogenes</i>	13040	Pan Fusarium	ND
<i>Listeria seeligeri</i>	35967D-5	Pan Fusarium	ND
<i>Listeria welshimeri</i>	35897D-5	Pan Fusarium	ND
<i>Listeria monocytogenes</i>	7647	Pan Fusarium	ND
<i>Salmonella</i>	700720	Pan Fusarium	ND
<i>Salmonella indica</i>	1578	Pan Fusarium	ND
<i>Staph aureus</i>	12600	Pan Fusarium	ND
<i>Yersinia pestis</i>	BAA-1511D-5	Pan Fusarium	ND
<i>Klebsiella pneumoniae</i>	BAA-2146	Pan Fusarium	ND
<i>Shigella flexneri</i>	29903D-5	Pan Fusarium	ND
<i>Pseudomonas aeruginosa</i>	35554	Pan Fusarium	ND
<i>Pseudomonas aeruginosa</i>	13525	Pan Fusarium	ND
<i>lactobacillus</i>	10075	Pan Fusarium	ND
<i>Pythium dissorocum</i>	Punja	Pan Fusarium	ND
<i>Pythium myriotylum</i>	Punja	Pan Fusarium	ND

*The Pan *Fusarium* Assay generates a positive result when *Aspergillus flavus* is present (*Aspergillus oryzae* is a sub-species of *Aspergillus flavus*). *Aspergillus flavus* and *Fusarium* are fungi that can both produce mycotoxins. To speciate between the two, it is necessary to run a separate *Aspergillus* detection assay such as the [PathoSEEK *Aspergillus flavus* Detection Assay](#).

REVISION HISTORY

Version	Date	Description
v1	September 2024	Updated <i>Fusarium</i> specific validation document

DISCLAIMER

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