Validation of *Aspergillus* Duplex-TaqMan qPCR Assay

BACKGROUND

Aspergillus is a genus of filamentous fungi widely distributed in soil, air, dust, and decaying organic matter. Several species are of particular concern in the cannabis industry due to their ability to contaminate cannabis flower and inhalable products, posing potential risks to human health. *Aspergillus* contamination has been documented across cultivation and post-harvest environments and remains a primary target of microbial safety testing in regulated markets[1]. While hundreds of *Aspergillus* species exist, only a subset is considered clinically significant and routinely monitored in cannabis testing programs[2]. The TUMI Genomics PCR assay targets the key species most commonly included in compliance testing:

Aspergillus fumigatus
Aspergillus niger
Aspergillus flavus / Aspergillus parasiticus
Aspergillus terreus

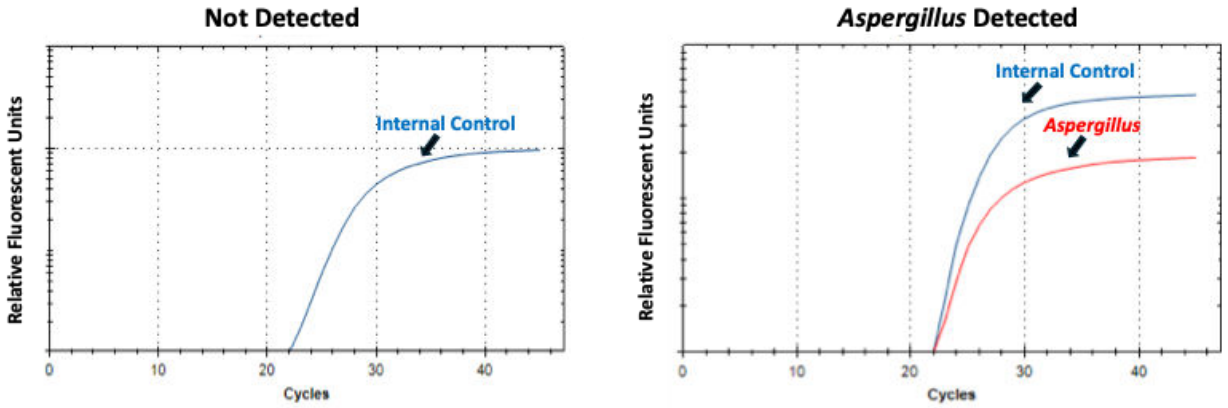
Although *Aspergillus* is commonly screened during state compliance testing, detection at the final product stage leaves limited options for remediation, often resulting in product disposal and significant economic loss. *Aspergillus* species produce abundant airborne spores (conidia) that readily disperse through air and dust, allowing contamination to occur at multiple stages, including cultivation, harvest, drying, trimming, and storage.

This creates a critical opportunity for earlier detection and intervention. Implementing a preventative screening strategy enables cultivators to identify *Aspergillus* pressure before it escalates, allowing mitigation steps to be taken prior to widespread contamination and batch loss.

The TUMI Genomics *Aspergillus* assay is designed as a proactive screening tool to be used before remediation and compliance testing. It supports monitoring of environmental systems, surfaces, and high-risk areas such as drying and processing spaces, while also helping identify potentially contaminated batches prior to submission for state testing. When integrated into key stages of production, preventative *Aspergillus* screening can significantly reduce the risk of product failures and associated financial losses.

GENERAL INFORMATION

TUMI Genomics' *Aspergillus* PCR assay involves a swab-based collection process and is intended for detection of the fungus in cannabis and hemp foliage/flower tissue and environmental contamination. This assay can be used to screen for *Aspergillus* on surfaces and in plant tissue. The test uses a duplex TaqMan qPCR to detect the presence of *Aspergillus* DNA sequences. The primer/probe sets target the *Aspergillus* genome as well as an internal control to ensure effective fungal lysis and sample integrity.



Amplification curves from extracts that are negative (left panel) and positive (right panel) for *Aspergillus*.

TECHNICAL TEST VALIDATION

Technical validation consists of experiments to determine the technical (in lab) limits and characteristics of the assay.

LIMIT OF DETECTION (LoD)

A limit of detection (LoD) study determines the sensitivity of a test. The LoD is defined as the lowest concentration of a pathogen that can be detected in ≥95% of true positive samples. LoD studies help characterize how well a test can identify low-level or early infections.

The LoD for this assay (i.e., the lowest concentration of *Aspergillus* reliably detected) was determined by testing serial dilutions (1,000,000 copies to 2 copies) of *Aspergillus* genomic DNA prepared from each target species, with 10 replicates per concentration.

The lowest concentration reliably detected by the assay was determined to be approximately 7.6 *Aspergillus* cells per reaction. The LoD was then further confirmed by testing 30 replicates at decreasing concentrations near the assumed LoD for each species targeted by the assay. For each individual species the LoD ranged between 7-15 *Aspergillus* genome copies per reaction.

The table below summarizes the results used to determine the LoD (i.e., the lowest level of pathogen the assay can detect). The LoD is highlighted in green for each species.

Limit Of Detection – Broad Range

Sample Concentration (per reaction)	*Fraction Positive*	*Mean CT Internal Control*	*Standard Deviation*	*Mean CT Aspergillus*	*Standard Deviation*
1 X10^6 copies	10/10	28.57	0.25	19.30	1.54
5 X10^5 copies	10/10	28.49	0.18	19.76	1.77
2.5 X10^5 copies	10/10	28.51	0.19	20.88	1.70
1.25 X10^5 copies	10/10	28.28 in	0.32	21.72	1.65
62500 copies	10/10	28.34	0.17	22.89	1.70
31300 copies	10/10	28.34	0.13	23.83	1.64
15625 copies	10/10	28.35	0.15	24.76	1.70
7813 copies	10/10	28.41	0.14	25.71	1.52
39067 copies	10/10	28.49	0.08	26.75	1.51
1953 copies	10/10	28.43	0.17	27.57	1.41
977 copies	10/10	28.50	0.16	28.58	1.29
488 copies	10/10	28.43	0.15	29.35	1.19
244 copies	10/10	28.61	0.19	30.25	1.18
122 copies	10/10	28.47	0.20	30.84	1.22
61 copies	10/10	28.67	0.19	31.83	1.16
31 copies	10/10	28.58	0.19	32.70	1.37
15 copies	10/10	28.47	0.19	32.89	2.06
7.6 copies	**10/10**	**28.43**	**0.12**	**33.90**	**1.11**
3.8 copies	4/10	28.44	0.18	36.62	3.24
1.9 copies	3/10	28.47	0.09	37.88	5.11
0 copies	0/10	28.42	0.08	Not Detected	-

Limit of Detection – Narrow Range, Species Specific

Species	*Sample Concentration (per Reaction)*	*Fraction Positive*	*Percent Detection*	*Mean CT Aspergillus*	*Standard Deviation*
A. Niger	60 copies	30/30	100%	27.30	1.04
	30 copies	30/30	100%	28.95	1.13
	15 copies	**29/30**	**97%**	**30.52**	**2.38**
	7 copies	28/30	93%	31.32	2.02
	3.8 copies	26/30	87%	32.69	2.86
	1.9 copies	15/30	50%	28.95	1.13
	0 copies	0/30	0%	Not Detected	-

A. Flavus	60 copies	30/30	100%	27.92	1.20
	30 copies	30/30	100%	29.04	2.03
	15 copies	30/30	100%	29.66	1.03
	7 copies	**30/30**	**100%**	**30.67**	**0.89**
	3.8 copies	27/30	90%	31.20	1.32
	1.9 copies	28/30	93%	32.20	2.32
	0 copies	0/30	0%	Not Detected	-
A. Fumigatus	60 copies	30/30	100%	30.85	0.32
	30 copies	30/30	100%	31.93	0.38
	15 copies	29/30	97%	33.37	0.86
	7 copies	**29/30**	**97%**	**34.14**	**0.66**
	3.8 copies	28/30	93%	35.65	1.44
	1.9 copies	26/30	87%	36.19	1.23
	0 copies	0/30	0%	Not Detected	-
A. Terreus	60 copies	30/30	100%	30.24	0.95
	30 copies	30/30	100%	31.56	0.93
	15 copies	**29/30**	**97%**	**32.87**	**1.27**
	7 copies	25/30	83%	33.16	1.66
	3.8 copies	18/30	60%	34.04	2.31
	1.9 copies	9/30	30%	35.74	3.38
	0 copies	0/30	0%	Not Detected	-

CROSS-REACTIVITY

Cross-reactivity analysis evaluates the specificity of a test, whether the assay detects only the target pathogen or generates a signal due to non-target organisms. A highly specific test should demonstrate high specificity for the intended target and low similarity to potential non-target organisms (e.g., other fungi, oomycetes, or common plant-associated microbes).

Cross-reactivity was evaluated using an *in silico* analysis of the assay primer and probe sequences against genomic sequences from microorganisms commonly associated with the roots, stems, and leaves of *Cannabis sativa* (cannabis/hemp) and *Humulus lupulus* (hops). Genomic sequences were obtained from the National Center for Biotechnology Information (NCBI**)** database, and alignments were performed using the Basic Local Alignment Search Tool (BLAST)**.**

For each organism evaluated, percent cross-reactivity was calculated as:

Percent similarity = (number of matching nucleotides across primer/probe sequences) ÷ (total nucleotides in the Aspergillus primer/probe set)

Cross-Reactivity Results

Significant cross-reactivity was defined as >80% similarity between the primer/probe set and any sequence within a tested organism. Across the 343 microorganisms analyzed, only two organisms demonstrated a potential cross-reactivity and no cross-reactivity was observed within the cannabis genome. Of the two potentially interfering species, *Aspergillus oryzae* and *Aspergillus paraciticus*, neither are likely to be a significant concern for the intended use of this PCR assay.

A. oryzae is a domesticated fungal species used within controlled industrial fermentation processes in East Asia for the production of food products including; sake, soy sauce, and miso[3]. *A. oryzae is* not commonly found in natural/agricultural environments and is poorly adapted to survival outside of human engineered cultures, making its presence in cannabis cultivation or indoor grow facilities extremely unlikely.

A. parasiticus, while more environmentally relevant, is closely related to *A. flavus* and is similarly capable of producing aflatoxins and acting as an opportunistic human pathogen[4]. As such, its detection would not represent a problematic false positive in the context of cannabis safety testing, but rather a meaningful indication of a potentially hazardous contaminant.

Taken together, the observed cross-reactivity does not materially impact the specificity or practical utility of the assay for application in cannabis cultivation operations.

Table Summary

The table below lists each microorganism evaluated, total number of sequence variants analyzed per species, and the corresponding percent similarity to the *Aspergillus* primer/probe sets indicated. Organisms highlighted in red have >80% similarity and were investigated further.

	Percent Cross-Reactivity			
Species Analyzed	***Niger***	***Flavus***	***Fumigatus***	***Terreus***
Aspergillus_niger	**100.00%**	34.29%	39.39%	36.92%
Aspergillus_flavus	40.91%	**100.00%**	40.91%	41.54%
Aspergillus_fumigatus	40.91%	38.57%	**100.00%**	47.69%
Aspergillus_terreus	39.39%	41.43%	43.94%	**100.00%**
Cannabis_sativa	**34.85%**	**34.29%**	**42.42%**	**35.38%**
Aspergillus_oryzae	40.91%	**98.57%**	39.39%	41.54%
Aspergillus_parasiticus	40.91%	**98.57%**	40.91%	36.92%
Trichoderma_harzianum	63.64%	57.14%	65.15%	41.54%
Trichosporon ovoides	59.09%	57.14%	45.45%	43.08%
Fusarium_brachygibbosum	43.94%	57.14%	34.85%	35.38%
Schizosaccharomyces cryophilus	34.85%	57.14%	34.85%	35.38%
Hydnopolyporus_fimbriatus	36.36%	52.86%	39.39%	36.92%

Holtermannia corniformis	36.36%	51.43%	43.94%	36.92%
Phytophthora_capsici	45.45%	50.00%	45.45%	36.92%
Cryptococcus_laurentii	40.91%	48.57%	40.91%	35.38%
Aspergillus_carbonarius	37.88%	48.57%	40.91%	40.00%
Kwoniella botswanensis	37.88%	48.57%	42.42%	38.46%
Trichosporon laibachii	66.67%	47.14%	48.48%	36.92%
Rhodotorula mucilaginosa	48.48%	47.14%	46.97%	30.77%
Mucor_circinelloides	36.36%	47.14%	36.36%	36.92%
Fusarium_proliferatum	45.45%	45.71%	42.42%	38.46%
Fusarium_vanettenii	36.36%	45.71%	36.36%	43.08%
Fusarium_mundagurra	34.85%	45.71%	30.30%	27.69%
Tremella fuciformis	34.85%	45.71%	34.85%	41.54%
Filobasidium globisporum	33.33%	45.71%	33.33%	33.85%
Malassezia vespertilionis	33.33%	45.71%	33.33%	33.85%
Fusarium_avenaceum	39.39%	44.29%	36.36%	36.92%
Piskurozyma capsuligena	39.39%	44.29%	39.39%	32.31%
Fusarium_sp._VM40	36.36%	44.29%	40.91%	36.92%
Kwoniella heveanensis	33.33%	44.29%	40.91%	46.15%
Malassezia caprae	30.30%	44.29%	30.30%	30.77%
Cryptococcus phenolicus	42.42%	42.86%	40.91%	46.15%
Pythium_aphanidermatum	40.91%	42.86%	48.48%	33.85%
Cryptococcus curvatus	39.39%	42.86%	43.94%	40.00%
Aspergillus_nidulans	36.36%	42.86%	40.91%	40.00%
Cryptococcus bacillisporus	36.36%	42.86%	36.36%	36.92%
Cryptococcus decagattii	36.36%	42.86%	36.36%	36.92%
Cryptococcus skinneri	34.85%	42.86%	42.42%	35.38%
Taphrina populina	30.30%	42.86%	31.82%	30.77%
Fusarium_oxysporum	62.12%	41.43%	68.18%	40.00%
Metarhizium_anisopliae	45.45%	41.43%	36.36%	49.23%
Diaporthe_eres	42.42%	41.43%	36.36%	41.54%
Fusarium_commune	42.42%	41.43%	36.36%	46.15%
Fusarium_sp._MPI-SDFR-AT-0072	42.42%	41.43%	36.36%	36.92%
Cladosporium	40.91%	41.43%	36.36%	40.00%
Fusarium_piperis	40.91%	41.43%	36.36%	36.92%
Trichosporon inkin	40.91%	41.43%	42.42%	33.85%
Cutaneotrichosporon dermatis	39.39%	41.43%	45.45%	40.00%
Aspergillus_tubingensis	36.36%	41.43%	40.91%	36.92%
Taphrina flavorubra	33.33%	41.43%	33.33%	33.85%

Trichosporon gracile	46.97%	40.00%	69.70%	43.08%
Trichosporon gamsii	43.94%	40.00%	42.42%	36.92%
Penicillium_rubens	40.91%	40.00%	36.36%	50.77%
Trichosporon faecale	40.91%	40.00%	43.94%	44.62%
Aspergillus_fijiensis	39.39%	40.00%	30.30%	30.77%
Aspergillus_versicolor	39.39%	40.00%	36.36%	44.62%
Taphrina deformans	39.39%	40.00%	53.03%	41.54%
Beauveria_bassiana	36.36%	40.00%	42.42%	36.92%
Fusarium_graminearum	36.36%	40.00%	42.42%	36.92%
Fusarium_vorosii	36.36%	40.00%	36.36%	36.92%
Penicillium_chrysogenum	36.36%	40.00%	40.91%	50.77%
Cryptococcus wieringae	34.85%	40.00%	34.85%	35.38%
Cystofilobasidium bisporidii	34.85%	40.00%	34.85%	35.38%
Fusarium_nematophilum	62.12%	38.57%	42.42%	36.92%
Naganishia kalamii	62.12%	38.57%	36.36%	36.92%
Mrakia aquatica	45.45%	38.57%	40.91%	41.54%
Pythium_oligandrum	45.45%	38.57%	43.94%	36.92%
Microbotryum violaceum	43.94%	38.57%	45.45%	38.46%
Cystofilobasidium capitatum	42.42%	38.57%	36.36%	36.92%
Fusarium_flagelliforme	42.42%	38.57%	39.39%	36.92%
Fusarium_musae	42.42%	38.57%	40.91%	40.00%
Holtermanniella nyarrowii	42.42%	38.57%	36.36%	36.92%
Apiotrichum loubieri	40.91%	38.57%	42.42%	40.00%
Cryptococcus terricola	40.91%	38.57%	40.91%	40.00%
Glaciozyma martinii	40.91%	38.57%	40.91%	41.54%
Pythium_insidiosum	40.91%	38.57%	34.85%	35.38%
Pythium_sp._B7052-1	40.91%	38.57%	43.94%	40.00%
Cryptococcus albidus	37.88%	38.57%	42.42%	38.46%
Erythrobasidium hasegawianum	36.36%	38.57%	36.36%	36.92%
Glaciozyma antarctica	36.36%	38.57%	39.39%	36.92%
Nigrospora_oryzae	36.36%	38.57%	43.94%	41.54%
Penicillium_polonicum	36.36%	38.57%	42.42%	46.15%
Pythium_dissotocum	36.36%	38.57%	59.09%	46.15%
Tremella mesenterica	36.36%	38.57%	40.91%	36.92%
Vanrija humicola	36.36%	38.57%	43.94%	36.92%
Taphrina wiesneri	33.33%	38.57%	33.33%	33.85%
Schizosaccharomyces japonicus	62.12%	37.14%	34.85%	35.38%
Rhodotorula sphaerocarpa	50.00%	37.14%	40.91%	40.00%

Naganishia antarctica	48.48%	37.14%	45.45%	36.92%
Trichosporon coremiiforme	48.48%	37.14%	42.42%	40.00%
Trichosporon veenhuisii	46.97%	37.14%	42.42%	40.00%
Udeniomyces pyricola	46.97%	37.14%	40.91%	33.85%
Filobasidium uniguttulatum	45.45%	37.14%	37.88%	35.38%
Alternaria_tenuissima	43.94%	37.14%	45.45%	38.46%
Bulleromyces albus	43.94%	37.14%	45.45%	33.85%
Fusarium_haematococcum	43.94%	37.14%	36.36%	36.92%
Fusarium_solani	43.94%	37.14%	39.39%	40.00%
Fusarium_solani-melongenae	43.94%	37.14%	65.15%	40.00%
Trichosporon brassicae	43.94%	37.14%	39.39%	40.00%
Trichosporon guehoae	43.94%	37.14%	45.45%	36.92%
Malassezia dermatis	42.42%	37.14%	30.30%	29.23%
Trichosporon asahii	42.42%	37.14%	31.82%	40.00%
Vanrija fragicola	42.42%	37.14%	39.39%	40.00%
Yarrowia_lipolytica	42.42%	37.14%	39.39%	40.00%
Cutaneotrichosporon cyanovorans	40.91%	37.14%	39.39%	40.00%
Cutaneotrichosporon daszewskae	40.91%	37.14%	42.42%	40.00%
Fusarium_irregulare	40.91%	37.14%	46.97%	36.92%
Rhizoctonia_solani	40.91%	37.14%	42.42%	36.92%
Tremella yokohamensis	40.91%	37.14%	48.48%	40.00%
Apiotrichum cacaoliposimile	39.39%	37.14%	45.45%	40.00%
Aspergillus_brasiliensis	39.39%	37.14%	42.42%	36.92%
Cutaneotrichosporon arboriformis	39.39%	37.14%	42.42%	40.00%
Cutaneotrichosporon mucoides	39.39%	37.14%	46.97%	40.00%
Fusarium_sp._FIESC_RH6	39.39%	37.14%	45.45%	36.92%
Kwoniella pini	39.39%	37.14%	39.39%	33.85%
Malassezia brasiliensis	39.39%	37.14%	39.39%	40.00%
Malassezia nana	39.39%	37.14%	39.39%	43.08%
Meredithblackwellia eburnea	39.39%	37.14%	39.39%	40.00%
Trichothecium_roseum	39.39%	37.14%	34.85%	44.62%
Kwoniella europaea	37.88%	37.14%	42.42%	38.46%
Cryptococcus neoformans	36.36%	37.14%	36.36%	36.92%
Cryptococcus_neoformans	36.36%	37.14%	36.36%	36.92%
Malassezia equina	36.36%	37.14%	36.36%	36.92%
Cryptococcus flavescens	34.85%	37.14%	34.85%	40.00%
Epicoccum_nigrum	34.85%	37.14%	42.42%	35.38%
Kwoniella bestiolae	34.85%	37.14%	39.39%	35.38%

Schizosaccharomyces octosporus	34.85%	37.14%	34.85%	35.38%
Schizosaccharomyces pombe	34.85%	37.14%	34.85%	35.38%
Cryptococcus depauperatus	33.33%	37.14%	40.91%	33.85%
Cryptococcus fagi	33.33%	37.14%	31.82%	32.31%
Rhizopus	30.30%	37.14%	30.30%	49.23%
Escherichia_coli	28.79%	37.14%	30.30%	29.23%
Cutaneotrichosporon cavernicola	65.15%	35.71%	42.42%	38.46%
Taphrina confusa	45.45%	35.71%	33.33%	33.85%
Fusarium_sambucinum	43.94%	35.71%	37.88%	38.46%
Fusarium_equiseti	42.42%	35.71%	40.91%	36.92%
Schizosaccharomyces versatilis	42.42%	35.71%	39.39%	35.38%
Fusarium_flocciferum	39.39%	35.71%	39.39%	38.46%
Cryptococcus neoformans	37.88%	35.71%	46.97%	38.46%
Fusarium_albosuccineum	37.88%	35.71%	37.88%	38.46%
Krasilnikovozyma curviuscula	37.88%	35.71%	37.88%	38.46%
Malassezia arunalokei	37.88%	35.71%	37.88%	41.54%
Savitreella phatthalungensis	37.88%	35.71%	39.39%	38.46%
Sporobolomyces carnicolor	37.88%	35.71%	48.48%	38.46%
Takashimella tepidaria	37.88%	35.71%	37.88%	38.46%
Cryptococcus gattii	36.36%	35.71%	36.36%	36.92%
Mucor	36.36%	35.71%	36.36%	36.92%
Udeniomyces megalosporus	36.36%	35.71%	43.94%	32.31%
Acinetobacter_baumanii	21.21%	35.71%	27.27%	21.54%
Apiotrichum domesticum	63.64%	34.29%	72.73%	36.92%
Fusarium_phialophorum	62.12%	34.29%	68.18%	36.92%
Rhodosporidiobolus nylandii	46.97%	34.29%	39.39%	36.92%
Trichoderma	46.97%	34.29%	48.48%	36.92%
Aspergillus_steynii	43.94%	34.29%	45.45%	40.00%
Fusarium_euwallaceae	43.94%	34.29%	36.36%	36.92%
Fusarium_keratoplasticum	43.94%	34.29%	45.45%	36.92%
Fusarium_kuroshium	43.94%	34.29%	36.36%	36.92%
Fusarium_oligoseptatum	43.94%	34.29%	42.42%	36.92%
Fusarium_phalaenopsidis	43.94%	34.29%	39.39%	36.92%
Fusarium_sp._AF-6	43.94%	34.29%	36.36%	36.92%
Fusarium_sp._Ph1	43.94%	34.29%	45.45%	36.92%
Fusarium_torreyae	43.94%	34.29%	36.36%	44.62%
Mortierella_hyalina	43.94%	34.29%	36.36%	40.00%
Naganishia adeliensis	43.94%	34.29%	36.36%	36.92%

Naganishia liquefaciens	43.94%	34.29%	36.36%	36.92%
Papiliotrema terrestris	42.42%	34.29%	36.36%	36.92%
Phenoliferia glacialis	42.42%	34.29%	45.45%	36.92%
Rhodotorula taiwanensis	42.42%	34.29%	45.45%	36.92%
Tausonia pamirica	42.42%	34.29%	42.42%	36.92%
Trichosporon cutaneum	42.42%	34.29%	46.97%	36.92%
Dioszegia takashimae	40.91%	34.29%	36.36%	36.92%
Penicillium_expansum	40.91%	34.29%	36.36%	36.92%
Phenoliferia psychrophenolica	40.91%	34.29%	45.45%	36.92%
Tausonia pullulans	40.91%	34.29%	36.36%	36.92%
Aspergillus_glaucus	39.39%	34.29%	42.42%	36.92%
Fusarium_luffae	39.39%	34.29%	46.97%	36.92%
Kwoniella shandongensis	39.39%	34.29%	36.36%	36.92%
Trichosporon montevideense	39.39%	34.29%	63.64%	41.54%
Alternaria	36.36%	34.29%	36.36%	36.92%
Alternaria_alternata	36.36%	34.29%	36.36%	36.92%
Aspergillus_aculeatus	36.36%	34.29%	36.36%	36.92%
Aspergillus_alabamensis	36.36%	34.29%	36.36%	96.92%
Aspergillus_carneus	36.36%	34.29%	36.36%	60.77%
Aspergillus_clavatus	36.36%	34.29%	36.36%	36.92%
Aspergillus_deflectus	36.36%	34.29%	36.36%	36.92%
Aspergillus_fischeri	36.36%	34.29%	78.48%	36.92%
Aspergillus_ochraceus	36.36%	34.29%	36.36%	36.92%
Aspergillus_pseudoterreus	36.36%	34.29%	36.36%	78.46%
Aspergillus_tamarii	36.36%	34.29%	39.39%	36.92%
Cercospora_canescens	36.36%	34.29%	36.36%	36.92%
Chaetomium_brasiliensis	36.36%	34.29%	36.36%	36.92%
Chaetomium_elatum	36.36%	34.29%	36.36%	36.92%
Cryptococcus deuterogattii	36.36%	34.29%	36.36%	36.92%
Cryptococcus sp	36.36%	34.29%	43.94%	36.92%
Cryptococcus tetragattii	36.36%	34.29%	36.36%	40.00%
Cystofilobasidium macerans	36.36%	34.29%	39.39%	36.92%
Dioszegia aurantiaca	36.36%	34.29%	42.42%	43.08%
Dioszegia crocea	36.36%	34.29%	36.36%	44.62%
Erysiphe_necator	36.36%	34.29%	36.36%	36.92%
Fusarium_agapanthi	36.36%	34.29%	36.36%	36.92%
Fusarium_ananatum	36.36%	34.29%	36.36%	36.92%
Fusarium_andiyazi	36.36%	34.29%	36.36%	36.92%

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INCLUSIVITY

An inclusivity analysis evaluates how well a test detects known sequence variants of a target pathogen. An acceptable inclusivity analysis demonstrates that the assay is designed to reliably detect all, or the vast majority, of known variants of the target organism(s).

To assess assay inclusivity, all primer and probe sequences were cross-referenced against known sequence variants for each targeted *Aspergillus* species. Primers and probes were intentionally designed to bind a highly conserved region of the *Aspergillus* genome to maximize detection across diverse strains and variants, while also detecting sequences unique to each of the four targeted species to provide maximum specificity.

Inclusivity Results

A total of 1,524 unique sequence variants were analyzed across four pathogenic *Aspergillus* species. Among these variants, 99.3% showed 100% homology, and 100% showed >95% homology to the primer/probe sequences used in the assay.

None of the analyzed sequences contained more than a single SNP within any individual primer or probe. These results indicate that the assay is likely to detect the vast majority of known *Aspergillus* sequence variants targeted by this assay.

Table Summary

The table below summarizes the number of sequences analyzed for each targeted *Aspergillus* species and the percent homology of the primer/probe set across all sequences evaluated. As shown, the primer and probe sets matched all known *Aspergillus* sequence variants with >95% homology.

	Sequences Analyzed	*100% Primer/Probe match*	*>95% primer match*	*SNPs in Multiple Primers?*
A. niger	213	100%	-	No
A. flavus	513	98.8%	100%	No
A. fumigatus	622	99.7%	100%	No
A.terreus	176	99.4%	100%	No

TECHNICAL ACCURACY

A technical accuracy test determines the accuracy of an assay compared to an alternative, high sensitivity test. An acceptable technical accuracy shows at least 95% agreement between results obtained from the assay in question and a comparison method.

To determine technical accuracy, 90 *Aspergillus* positive samples with variable levels and 30 *Aspergillus* negative samples were prepared. Samples incubated at room temperature for two days to mimic standard shipping times from customer facilities to the TUMI Genomics laboratory. Samples were then coded and tested by an independent, blinded operator using TUMI Genomics *Aspergillus* qRT-PCR assay. The presence/absence of *Aspergillus* in the sample was confirmed via plating.

As shown by the table below, **the TUMI Genomics *Aspergillus* qRT-PCR assay performed with an overall 99.2% accuracy.** The test detected 98.9% of the *Aspergillus* (+) samples down to ~one colony forming unit per test, confirming an expected sensitivity of 1 colony forming unit for the assay. No false positive results were obtained.

Sample	Validation Intent	Target CFUs per test	Probability input ≥ 1 CFU	Probability input contains 0 CFUs	95 % Poisson CFU Range per Test	Fraction Aspergillus Detected via PCR	Accuracy
Low-*Asp* +	Minimum level expected to contain 1 CFU with 95% confidence	3	95%	5%	1-5*	29/30	96.7%
Mid-*Asp* +	Intermediate Input Level	25	100%	~0%	16-35	30/30	100%
High-*Asp* +	High Input Level	100	100%	~0%	81-120	30/30	100%
Negative	False Positive Determination	0	0%	~100%	NA	0/30	100%
Final Accuracy Compared to Plating						**99.2%**	

*** The low-input dilution (Low-Asp +) was defined as the minimum concentration expected to provide at least 1 CFU per test aliquot with 95% confidence. Under a Poisson model, this corresponds to a target mean of 3 CFUs per test aliquot.**

TUMI Genomics Lead Scientists

Tassa Saldi, PhD: Dr. Saldi received her undergraduate and graduate degrees in molecular biology from the University of Colorado in Boulder and completed her post-doctoral studies at the Health Sciences Center, University of Colorado, Denver. Her graduate work explored the molecular mechanism underpinning Amyotrophic Lateral Sclerosis (ALS) and the role of double-stranded RNA accumulation and heterochromatin in pathogenesis. Continuing her work on structured RNA during post-doctoral work, Dr. Saldi investigated the role of genome-wide nascent RNA secondary structure in co-transcriptional splicing, A-to-I RNA editing and transcription termination. Her work was supported by fellowships from the American Cancer Society and the RNA Biosciences Intuitive (RBI). Following her postdoc, Dr. Saldi directed the COVID-19 surveillance lab at CU, Boulder where she supervised a team of 8 scientists and designed and validated multiple PCR assays to detect SARS-CoV-2 in human saliva. She is a lead scientist and CSO of TUMI Genomics.

Her publications can be found here: https://pubmed.ncbi.nlm.nih.gov/?term=Tassa+Saldi&sort=date

Alfonso Garrido-Lecca, PhD: Dr. Garrido-Lecca received an undergraduate degree in biology with a minor in chemistry from Texas A&M University. He pursued his PhD at the University of Colorado, Boulder in molecular biology. His graduate work focused on using the unique genetic organization of *C. elegans* to understand how genes are expressed and RNA transcripts processed. His postdoctoral work focused on the regulation of microRNAs in leukemia and was supported by a fellowship from the Linda Crnic Institute for Down Syndrome and the National Institute of Health T32 training grant. Dr. Garrido-Lecca is a lead scientist at TUMI Genomics and head of Research and Development.

His publications can be found here:
https://pubmed.ncbi.nlm.nih.gov/?term=alfonso+garrido-lecca&sort=date

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