Hindawi
Advanced Gut & Microbiome Research
Volume 2022, Article ID 5143833, 9 pages
https://doi.org/10.1155/2022/5143833

Research Article

Analytical Validation of TaqMan™ Assays on the OpenArray™ Platform Using Applied Biosystems QuantStudio™ 12K Flex for the Rapid Identification of Nail Microbiota

Irfan A. Khan,[1,2] **Ishrat I. Ali**,[3] **Saeed Khan**,[4] **and Sagheer Ahmed**[5]

[1]*Department of Chemistry, University at Albany, State University of New York, Albany, New York, USA*
[2]*Suretox Laboratory, Elmwood Park, New Jersey, USA*
[3]*University of Karachi, Karachi, Pakistan*
[4]*Dow University of Health Sciences, Karachi, Pakistan*
[5]*Shifa Tameer-e-Millat University, Islamabad, Pakistan*

Correspondence should be addressed to Irfan A. Khan; ikhan3@albany.edu

Received 2 October 2022; Revised 30 October 2022; Accepted 31 October 2022; Published 15 November 2022

Academic Editor: Jiong Yu

Several microorganisms are known to play a role in nail infections. For the successful treatment of nail microbiota, especially fungi, there is a need for precise and robust diagnostic methods. This test utilizes real-time PCR amplification to detect the presence of a microorganism in a nail sample by amplifying the genomic DNA of the organism. Here, we described a TaqMan™-OpenArray™ nail microbiota assay that is efficient and easy-to-use for the characterization of key nail microbial targets through real-time PCR. The analytical accuracy and specificity of the nail panel were 100% and 99.90% across all assays and controls tested, respectively, and it was significantly more sensitive than culture. TaqMan™-OpenArray™ testing is fast and robust to contamination. In this paper, the main questions discussed were the replacement of culture by a broad-spectrum fungal TaqMan™-OpenArray™ testing and the implementation of TaqMan™-OpenArray™ testing into routine clinical laboratory settings.

1. Introduction

Chronic nail fungal and other pathogen infections may cause substantial morbidity and are costly to treat [1]. Diagnostic methods that utilize fungal/bacterial colonization/cultural identification can be time-consuming and may not completely illustrate all present pathogens due to the lack of sensitivity of the method created by the competition of multiple organisms in a single sample. TaqMan™-OpenArray™ testing (quantitative PCR, qPCR) has become an established method for rapid detection, quantification, and identification of microbial agents over the past decade [2]. TaqMan™-OpenArray™ testing uses increased sensitivity and specificity to support the more accurate diagnosis of crucial infections, or lack thereof, to properly treat chronic fungal infections with faster turnaround times [3]. The most critical advantage of TaqMan™-OpenArray™-based identification of nail pathogens is the ability to build in antibiotic/antifungal resistance genes by testing on the same panel as the fungal and bacterial targets to determine which antibiotics would be ineffective for complete healing for the patient. This design strategy allows for fewer samples to be run on the same OpenArray but gives the most complete picture of the best path to successful treatment in 24 to 48 hours from the time the sample arrives at the laboratory.

Nail fungal infections or onychomycosis are estimated to occur in over a billion people each year. It is the cause of roughly 50% of nail deformities and affects approximately 14-18% of adults. It is important to identify the pathology of the nail deformation and determine a proper treatment plan. Research is suggesting the rates of infection are increasing. Fungi can infect almost any part of the body; however, these infections are most commonly observed in the skin and nails, mainly on the foot. Anyone can acquire these infections, but the elderly and critically ill are often the most who commonly suffer. Real-

Table 1: List of assays.

Assay ID	Target name
AICSXMN	*Aspergillus niger*
AP326G9	*Trichosporon mucoides*
AP329KC	*Neoscytalidium dimidiatum*
AP47W2P	*Scytalidium hyalinum, Neoscytalidium hyalinum, Neoscytalidium dimidiatum*
AP7DTKZ	*Nocardia*
AP9HMYA	*Actinomyces israelii*
AP9HN97	*Arthroderma vanbreuseghemii* AKA *Trichophyton mentagrophytes*
APAAAT7	*Cryptococcus neoformans*
APAACG3	*Aspergillus fumigatus*
APCE439	*Malassezia furfur*
APCFAW4	*Acremonium strictum*
APDJY9N	*Trichophyton verrucosum*
APFVRD9	*Alternaria alternata*
APKA736	*Alternaria tenuissima*
APMF3XK	*Scopulariopsis brevicaulis*
APPRN34	*Meyerozyma guilliermondii*
APYMNR3	*Corynebacterium jeikeium*
Ba04230908_s1	*PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA (mecA)*
Ba04646259_s1	*Staphylococcus aureus*
Ba04932081_s1	*Pseudomonas aeruginosa*
Fn04646220_s1	*Candida tropicalis*
Fn04646221_s1	*Candida parapsilosis*
Fn04646233_s1	*Candida albicans*
Fn07921933_s1	*Trichophyton rubrum*
Fn07921937_s1	*Epidermophyton floccosum*
Fn07921946_s1	*Alternaria spp.*
Fn07921951_s1	*Trichophyton interdigitale, Trichophyton mentagrophytes, Trichophyton tonsurans*
Fn07921952_s1	*Curvularia lunata*
Fn07921967_s1	*Fusarium solani*
Fn07921974_s1	*Trichophyton tonsurans*
Fn07921976_s1	*Geotrichum candidum*
Fn07921982_s1	*Microsporum gypseum*
Ac00010014_a1	Xeno

time PCR-OpenArray™ testing is important because conditions of psoriasis and malignant melanoma can mimic the appearance of fungal infections [1]. Since some of the medications can have some side effects that impact organ function, this allows us to be sure we are accurately treating the right organisms.

Accurate and prompt identification of nail microbiota is the key to proper infection management for patients suffering from fungal and other pathogenic infections. Chronic nail infections often involve multiple organisms. The presence of these organisms is found extensively in the fungal group, which plays a substantial role in delayed healing, increased complications, and poor outcomes. Most skin fungal pathogens are colonized in both aerobic and anaerobic environments. Our real-time PCR-OpenArray™ test is ideal for the identification of these infections. Rapid identification of nail microbiota using real-time PCR-OpenArray™ testing is one of the advanced technologies we developed to get our patients back to their normal routines as quickly as possible [4]. The turnaround times for culture-based fungal/bacterial identification methods and antibiotic sensitivity testing average 5-30 days, the reduction that real-time PCR-OpenArray™ testing can offer (2-3 days less) [5].

We have painstakingly developed a TaqMan™ assay on the OpenArray™ platform, with a resistance option for rapid detection and speciation of fungi and bacteria most commonly observed to harm the foot and nails [6, 7]. Real-time PCR amplification was performed using TaqMan™ assays consisting of two PCR primers and one fluorescently labeled (FAM dye) probe which hybridizes to the target organism's genomic DNA. The assays are preloaded onto TaqMan™-OpenArray™ plates. The format of this OpenArray plate allows for three replicates to be run in parallel per plate for all targets. Each OpenArray can be used for running 23 samples and 1 control sample. All thirty-four targets can be seen in Table 1. The

Table 2: The validation experiment strategy.

OpenArray	Day	NTC	Synthetic control	gDNA	Exclusivity	Human gDNA	Extracted	Total	Comment
1	1	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
2	1	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
3	1	0	0	0	0	0	24	24	Plasmid
4	2	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
5	2	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
6	2	0	0	0	0	0	24	24	Plasmid
7	3	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
8	3	6	1	11	1	1	4	24	Positive controls, specificity controls, plasmid, organism pools, NTC
9	3	0	0	0	0	0	24	24	Plasmid
10	Any one day	3	18	0	0	0	0	21	Plasmid dilutions for linear range

Table 3: List of characterized genomic DNA controls.

Assay ID	Organism	Source	SKU	Lot #
APAACG3	*A. fumigatus*	ATCC	1022D-2	60049759
AICSXMN	*A. niger*	ATCC	6275D-2	6382835
Fn04646233_s1	*C. albicans*	ATCC	10231D-5	63215120
APAAAT7	*C. neoformans*	ATCC	MYA-565D-5	62296930
Fn04646221_s1	*C. parapsilosis*	ATCC	22019D-5	205
Fn04646220_s1	*C. tropicalis*	ATCC	750D-5	70010336
Fn07921982_s1	*M. gypseum*	ATCC	52055	58463882
Ba04932081_s1	*P. aeruginosa*	ATCC	27853D-5	70010563
Ba04230908_s1; Ba04646259_s1	*S. aureus*	ATCC	BAA-1556D-5	58216603
Fn07921951_s1; Fn07921974_s1; AP9HN97	*T. interdigitale*	ATCC	9533D-2	59238936
Fn07921933_s1	*T. rubrum*	ATCC	28188	62297038

Table 4: List of characterized genomic DNAs for exclusivity.

Actinomyces naeslundii	12104D	70001617
Aspergillus terreus	20542D-2	59213909
Aspergillus versicolor	11730D-2	59177341
Candida glabrata	MYA-2950D-5	63330675
Pichia kudriavzevii	14243_D1	70031934
Clavispora lusitaniae	42720D-5	70016315
Corynebacterium glucuronolyticum	51862_D2	70004006
Pseudomonas denitrificans	13867_D1	70010813
Pseudomonas syringae	11528_D1	70004003
Staphylococcus saprophyticus	15305D-5	63768338

overall reproducibility, which included day-to-day as well as operator-to-operator variation, was 99.91% across all assays tested. The precision across all but one assay and sample resulted in standard deviations within the recommended ranges for most samples (<1 across days and <0.5 within a day). The limit of detection (LOD) for most of the assays was 10,000-20,000 copies input to extraction. The linear dynamic range for all assays spanned at least 5 logs with an R^2 of >0.97 for most assays. Sample extractions of *C. albicans* and *S. aureus* were detected successfully after extraction in all replicates. Nail microbiota real-time PCR-OpenArray™ assay is fast and robust to contamination. This study is aimed at validating the practicability of a real-time PCR-OpenArray™ assay to rapidly detect *C. albicans*, dermatophytes, nondermatophyte molds, yeasts, and bacterial pathogens from specimens suspected to have nail fungal infections or onychomycosis [6].

2. Material and Methods

2.1. Sample Extractions. Pooled whole organism controls were extracted and analyzed to ensure the recovery of different DNA sources using the extraction protocol. The organisms chosen are well documented as being difficult to lyse

(*C. albicans* and *S. aureus*). The organism control was extracted and run on six separate OpenArray plates.

2.2. Equipment

(i) Applied Biosystems™ QuantStudio™ 12K Flex PCR system using TaqMan™-OpenArray™ plates

(ii) Applied Biosystems™ KingFisher Flex™ magnetic particle processor

2.3. Reagents/Consumables. Thermo Fisher Scientific reagents and consumables are specified in protocols and procedures.

2.4. Example of All Calculations Needed to Produce Interpretable Results. This is a qualitative test that detects the amplification of a specific target DNA sequence in an extracted sample. As such, the interpretable result is a "present/absent" call based on the evaluation of the quality of the amplification (Crt value) from each sample replicate using the metrics produced in the QuantStudio™ 12K Flex software. Data produced on the OpenArray™ plates are evaluated with the following criteria:

(1) Crt value is greater than 0 and less than 31

(2) Amp score is equal to or greater than 1.1

(3) Cq confidence score is equal to or greater than 0.7

At least 50% of the replicates for a sample must meet these criteria for the sample to be classified as "present" for the assay. Assays are spotted in triplicate and therefore 2 out of 3 replicates must meet the criteria. Manual calls may be made after inspection of the data and will be recorded as such.

3. Results

3.1. Validation Result Summary for Nail Microbiota Assays. This test was designed for high-throughput screening of several samples across a defined number of targets. In order to validate this qualitative test for microorganism identification, the following performance characteristics were evaluated:

(1) Analytical accuracy

(2) Analytical specificity

(3) Reproducibility

(4) Precision

(5) Limit of detection

(6) Linear dynamic range

As per laboratory procedures, testing includes inspection of all results. During the inspection, we determined that an unsuccessful or incorrect call can be corrected based on the manual examination of the amplification curve for that sample.

The validation experiments were carried out over 3 days, as follows (Table 2). All genomic DNA samples were run once on two OpenArray plates each day. Extractions were performed in triplicate, and each sample was run in duplicate on one OpenArray plate each day.

Table 5: The accuracy of the nail panel was 100% across all assays and controls tested.

Assay ID	Assay	Correct	Correct
APFVRD9	*A. alternata*	6	100.00%
APAACG3	*A. fumigatus*	12	100.00%
AP9HMYA	*A. israelii*	6	100.00%
AICSXMN	*A. niger*	12	100.00%
APCFAW4	*A. strictum*	6	100.00%
APKA736	*A. tenuissima*	6	100.00%
Fn07921946_s1	*Alternaria spp.*	6	100.00%
Fn04646233_s1	*C. albicans*	12	100.00%
APYMNR3	*C. jeikeium*	6	100.00%
Fn07921952_s1	*C. lunata*	6	100.00%
APAAAT7	*C. neoformans*	12	100.00%
Fn04646221_s1	*C. parapsilosis*	12	100.00%
Fn04646220_s1	*C. tropicalis*	12	100.00%
Fn07921937_s1	*E. floccosum*	6	100.00%
Fn07921967_s1	*F. solani*	6	100.00%
Fn07921976_s1	*G. candidum*	6	100.00%
APCE439	*M. furfur*	6	100.00%
APPRN34	*M. guilliermondii*	6	100.00%
Fn07921982_s1	*M. gypseum*	12	100.00%
Ba04230908_s1	*mecA*	12	100.00%
AP329KC	*N. dimidiatum*	6	100.00%
AP7DTKZ	*Nocardia*	6	100.00%
Ba04932081_s1	*P. aeruginosa*	12	100.00%
Ba04646259_s1	*S. aureus*	12	100.00%
APMF3XK	*S. brevicaulis*	6	100.00%
AP47W2P	*S. hyalinum*	6	100.00%
Fn07921951_s1	*T. interdigitale*	12	100.00%
AP9HN97	*T. mentagrophytes*	12	100.00%
AP326G9	*T. mucoides*	6	100.00%
Fn07921933_s1	*T. rubrum*	12	100.00%
Fn07921974_s1	*T. tonsurans*	6	100.00%
APDJY9N	*T. verrucosum*	6	100.00%
Ac00010014_a1	Xeno	18	100.00%
	Grand total	288	100.00%

3.1.1. Analytical Accuracy. Analytical accuracy was evaluated based on comparison to well-characterized, normalized genomic DNA, organism extraction, and multitarget synthetic DNA samples (Tables 3 and 4). The data is presented in Table 5. The accuracy across all assays and days was 100%.

3.1.2. Analytical Specificity. Analytical specificity was analyzed by examining potential cross-reactivity with common microflora-related species and cross-reactivity among targets within the panel using well-characterized, normalized genomic DNA pools (Tables 3 and 4). No template control (NTC) and negative extraction control (NEC) were evaluated for specificity in the absence of microbial DNA targets. These data are presented in Table 6. Assay specificity across all assays was 99.90%.

Table 6: The assay specificity of the nail panel was detecerrted at 99.90% across all assays and controls tested.

Assay ID	Assay	Correct	Correct	Wrong	Wrong
APFVRD9	*A. alternata*	96	100.00%		0.00%
APAACG3	*A. fumigatus*	90	100.00%		0.00%
AP9HMYA	*A. israelii*	96	100.00%		0.00%
AICSXMN	*A. niger*	90	100.00%		0.00%
APCFAW4	*A. strictum*	96	100.00%		0.00%
APKA736	*A. tenuissima*	96	100.00%		0.00%
Fn07921946_s1	*Alternaria spp.*	96	100.00%		0.00%
Fn04646233_s1	*C. albicans*	90	100.00%		0.00%
APYMNR3	*C. jeikeium*	96	100.00%		0.00%
Fn07921952_s1	*C. lunata*	96	100.00%		0.00%
APAAAT7	*C. neoformans*	87	96.67%	3	3.33%
Fn04646221_s1	*C. parapsilosis*	90	100.00%		0.00%
Fn04646220_s1	*C. tropicalis*	90	100.00%		0.00%
Fn07921937_s1	*E. floccosum*	96	100.00%		0.00%
Fn07921967_s1	*F. solani*	96	100.00%		0.00%
Fn07921976_s1	*G. candidum*	96	100.00%		0.00%
APCE439	*M. furfur*	96	100.00%		0.00%
APPRN34	*M. guilliermondii*	96	100.00%		0.00%
Fn07921982_s1	*M. gypseum*	90	100.00%		0.00%
Ba04230908_s1	*mecA*	90	100.00%		0.00%
AP329KC	*N. dimidiatum*	96	100.00%		0.00%
AP7DTKZ	*Nocardia*	96	100.00%		0.00%
Ba04932081_s1	*P. aeruginosa*	90	100.00%		0.00%
Ba04646259_s1	*S. aureus*	90	100.00%		0.00%
APMF3XK	*S. brevicaulis*	96	100.00%		0.00%
AP47W2P	*S. hyalinum*	96	100.00%		0.00%
Fn07921951_s1	*T. interdigitale*	90	100.00%		0.00%
AP9HN97	*T. mentagrophytes*	90	100.00%		0.00%
AP326G9	*T. mucoides*	96	100.00%		0.00%
Fn07921933_s1	*T. rubrum*	90	100.00%		0.00%
Fn07921974_s1	*T. tonsurans*	96	100.00%		0.00%
APDJY9N	*T. verrucosum*	96	100.00%		0.00%
Ac00010014_a1	Xeno	84	100.00%		0.00%
	Grand total	3075	99.90%	3	0.10%

The exclusivity sample was positive in three cases for *C neoformans* with Cq values > 29 (Table 6).

3.1.3. Reproducibility. Reproducibility was analyzed by comparing the "present" or "absent" classification of each call for a sample over a three-day period. The ratio of discordant calls to the total number of calls was used to determine the percentage of reproducibility. Data for reproducibility is presented in Table 7. This test has day-to-day and operator-to-operator reproducibility of 99.91% across all assays. The risk of incorrect calls will be mitigated by an examination of amplification data prior to reporting. Samples can be rerun when inspection of the data brings any results into question.

3.1.4. Precision. Precision was evaluated by calculating the standard deviation of Crt values of replicates within single arrays and across arrays used over a three-day period. All assays showed across day standard deviation ≤ 1 and within a day a standard deviation of ≤ 0.5 across all replicates.

3.1.5. Limit of Detection (LOD). LOD was evaluated using six different concentrations of multitarget plasmid control spiked into PBS plus nail and taken through extraction: 5000, 1500, 1000, and 500 copies/μl (20 μl of each was spiked into extraction). This resulted in a total of 100000, 30000, 20000, and 10000 copies per sample analyzed. These values correspond to theoretical amounts of 28, 8, 6, and 3 copies of DNA per through-hole, assuming an average 60 μl actual elution volume and 100% DNA recovery. Three extractions were performed at each concentration, and each sample was run on three separate OpenArray plates. A total of 18 calls were generated for each concentration across the three-day testing

Table 7: The overall reproducibility, which included day-to-day as well as operator-to-operator variation, was 99.91% across all assays tested.

Assay ID	Target organism	Total concordant	Total discordant	Reproducibility
APFVRD9	*A. alternata*	102		100.00%
APAACG3	*A. fumigatus*	102		100.00%
AP9HMYA	*A. israelii*	102		100.00%
AICSXMN	*A. niger*	102		100.00%
APCFAW4	*A. strictum*	102		100.00%
APKA736	*A. tenuissima*	102		100.00%
Fn07921946_s1	*Alternaria spp.*	102		100.00%
Fn04646233_s1	*C. albicans*	102		100.00%
APYMNR3	*C. jeikeium*	102		100.00%
Fn07921952_s1	*C. lunata*	102		100.00%
APAAAT7	*C. neoformans*	99	3	97.06%
Fn04646221_s1	*C. parapsilosis*	102		100.00%
Fn04646220_s1	*C. tropicalis*	102		100.00%
Fn07921937_s1	*E. floccosum*	102		100.00%
Fn07921967_s1	*F. solani*	102		100.00%
Fn07921976_s1	*G. candidum*	102		100.00%
APCE439	*M. furfur*	102		100.00%
APPRN34	*M. guilliermondii*	102		100.00%
Fn07921982_s1	*M. gypseum*	102		100.00%
Ba04230908_s1	*mecA*	102		100.00%
AP329KC	*N. dimidiatum*	102		100.00%
AP7DTKZ	*Nocardia*	102		100.00%
Ba04932081_s1	*P. aeruginosa*	102		100.00%
Ba04646259_s1	*S. aureus*	102		100.00%
APMF3XK	*S. brevicaulis*	102		100.00%
AP47W2P	*S. hyalinum*	102		100.00%
Fn07921951_s1	*T. interdigitale*	102		100.00%
AP9HN97	*T. mentagrophytes*	102		100.00%
AP326G9	*T. mucoides*	102		100.00%
Fn07921933_s1	*T. rubrum*	102		100.00%
Fn07921974_s1	*T. tonsurans*	102		100.00%
APDJY9N	*T. verrucosum*	102		100.00%
Ac00010014_a1	Xeno	102		100.00%
				99.91%

period (Table 8). The total number of calls made for each assay at each concentration was evaluated, and the concentration with ≥95.00% correct calls was designated as the LOD for the assay. Table 8 shows the detailed LOD results of each assay including the Crt average for the determined LOD. Most assays (24) showed a LOD of 10,000 copies as the extraction input, 7 assays showed a LOD of 20,000 copies, 1 assay showed 30,000 copies, and 1 assay showed a LOD of 100,000 copies input.

3.1.6. Linear Dynamic Range. Linear dynamic range was evaluated using multitarget plasmid control at starting concentrations of $1 \times 10^6 - 1 \times 10^1$ copies/μl input into the qPCR reaction. Samples were run each day. Crt values for assay replicate each day were averaged, and the averages were plotted against the log concentration of target copies (Table 8). Regression plots for each assay are included, along with the slope and correlation coefficient (R^2) calculated for the data generated. All assays showed linearity for at least 5 logs, and the assays had R^2 values > 0.97. Data for the linear dynamic range is presented in Figure 1. The green highlighted cells indicate the dilutions included in the calculations.

3.1.7. Sample Extractions. C. albicans was detected successfully after extraction in all replicates. *S. aureus* was detected in 16 of 18 sample runs. Data is presented in Table 9. The lack of detection in the two samples is very likely due to insufficient input of samples as the Crt values are around 29-30. An artificial Xeno DNA control was spiked into the samples at a concentration of 2×10^6 copies/sample and was successfully detected at an average Crt range of 18-23.

Table 8: The LOD for the assays was 10,000-20,000 copies input to extraction.

Assay ID	Assay	cp input into extraction	cp/μl (20 ul input)	cp/throughout (60 μl elution vol.)	Ct average
APFVRD9	*A. alternata*	10000	500	3	28.87
APAACG3	*A. fumigatus*	10000	500	3	30.27
AP9HMYA	*A. israelii*	20000	1000	6	29.78
AICSXMN	*A. niger*	10000	500	3	29.14
APCFAW4	*A. strictum*	100000	5000	28	28.36
APKA736	*A. tenuissima*	10000	500	3	29.92
Fn07921946_s1	*Alternaria spp.*	10000	500	3	30.01
Fn04646233_s1	*C. albicans*	10000	500	3	28.63
APYMNR3	*C. jeikeium*	10000	500	3	30.61
Fn07921952_s1	*C. lunata*	20000	1000	6	29.21
APAAAT7	*C. neoformans*	10000	500	3	29.66
Fn04646221_s1	*C. parapsilosis*	10000	500	3	28.78
Fn04646220_s1	*C. tropicalis*	10000	500	3	28.87
Fn07921937_s1	*E. floccosum*	20000	1000	6	28.66
Fn07921967_s1	*F. solani*	10000	500	3	29.34
Fn07921976_s1	*G. candidum*	10000	500	3	28.91
APCE439	*M. furfur*	10000	500	3	29.46
APPRN34	*M. guilliermondii*	30000	1500	8	28.07
Fn07921982_s1	*M. gypseum*	10000	500	3	30.31
Ba04230908_s1	*mecA*	20000	1000	6	27.16
AP329KC	*N. dimidiatum*	10000	500	3	27.21
AP7DTKZ	*Nocardia*	10000	500	3	29.09
Ba04932081_s1	*P. aeruginosa*	10000	500	3	29.29
Ba04646259_s1	*S. aureus*	10000	500	3	28.81
APMF3XK	*S. brevicaulis*	20000	1000	6	29.29
AP47W2P	*S. hyalinum*	10000	500	3	26.57
Fn07921951_s1	*T. interdigitale*	10000	500	3	30.11
AP9HN97	*T. mentagrophytes*	10000	500	3	28.26
AP326G9	*T. mucoides*	20000	1000	6	29.57
Fn07921933_s1	*T. rubrum*	10000	500	3	30.16
Fn07921974_s1	*T. tonsurans*	20000	1000	6	29.52
APDJY9N	*T. verrucosum*	10000	500	3	29.98
Ac00010014_a1	Xeno	10000	500	3	23.47

For two of the three organism pools, nail pieces were added to the sample during extraction. The same was done with one of the two NEC samples. Comparison of the Crt values between samples containing nail pieces and those without did not indicate any inhibition of the PCR reaction.

4. Discussion

This report describes a methodology that allows the simultaneous detection of 34 nail fungal/bacterial targets in a single reaction. As fungal species identification is not routinely performed prior to microscopic examination and culture, the TaqMan™-OpenArray™ can be useful in clinical use. The current method, which has high sensitivity and a short turnaround time, can speed up the diagnosis of onychomycosis which is caused by dermatophytes, nondermatophyte fungi, and other yeasts and bacterial pathogens, thus having the potential to improve onychomycosis outcomes. The performance characteristics of this assay have been excellent including its analytical accuracy, analytical specificity, reproducibility, precision, limit of detection, and linear dynamic range.

To the best of our knowledge, this is the first report which describes a TaqMan™-OpenArray™ testing methodology for the detection of nail pathogens. This methodology presented herein allows nail pathogen detection quite rapidly because post-TaqMan™-OpenArray™ testing data analysis steps are not required. Moreover, the chances of residual contaminations which can lead to erroneous positive results are diminished. Our results reveal that the addition of real-time PCR-OpenArray™ testing in routine laboratory workflow for nail specimens could thoroughly augment the detection of dermatophytes *Trichophyton rubrum*, *T. mentagrophytes*, and *E. floccosum* and nondermatophyte fungi such as *Acremonium spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Scopulariopsis brevicaulis*, *C.*

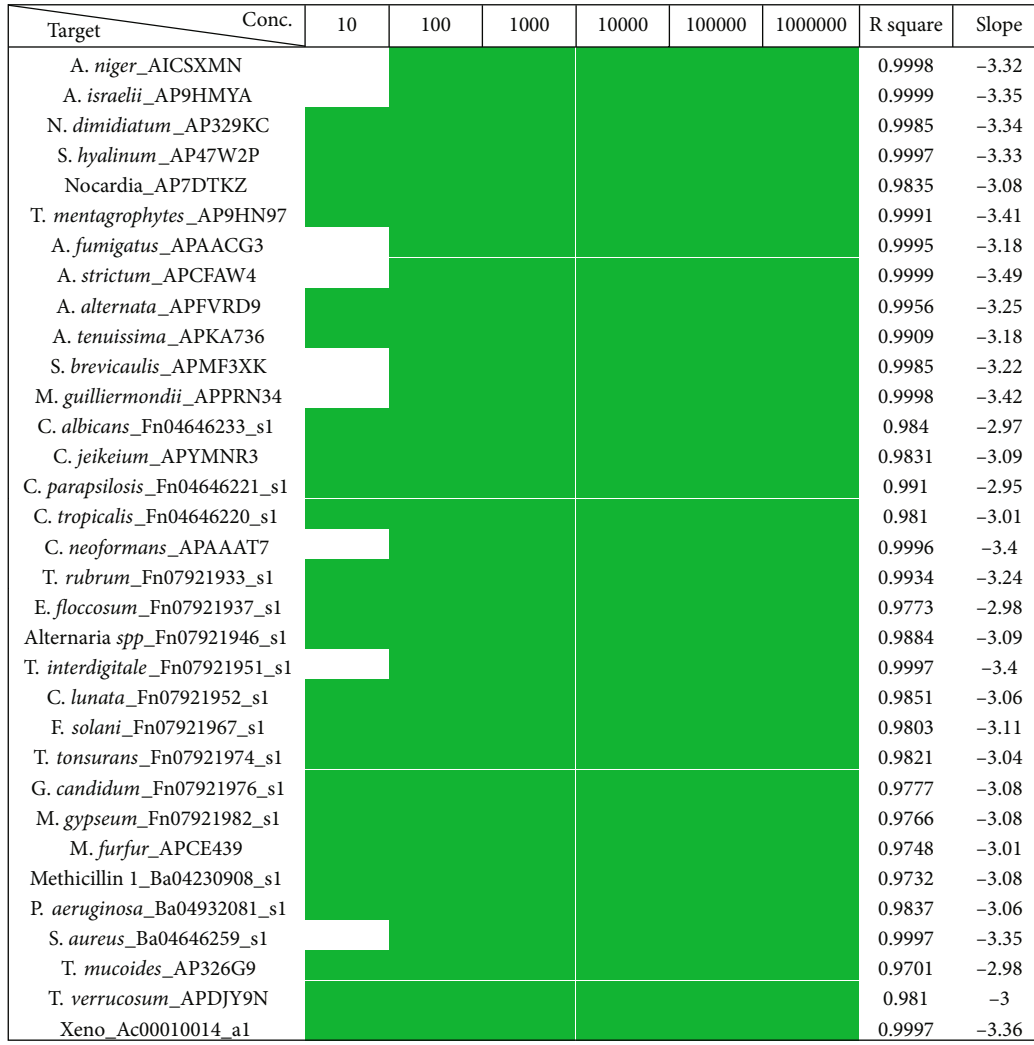

Figure 1: The linear dynamic range for all assays spanned at least 5 logs with an R^2 of >0.97 for most assays.

Table 9: *C. albicans* and *S. aureus* were detected successfully after extraction in all replicates.

Count of present/ absent	Column labels				
	C. albicans		*S. aureus*		Xeno
Row labels	Present	Absent	Present	Absent	Present
Orgpool A nail	6		5	1	6
Orgpool B nail	6		6		6
Orgpool C	6		5	1	6
NEC A nail		6		6	6
NEC B		6		6	6

albicans, and *C. parapsilosis* and other yeasts and bacterial pathogens such as *S. aureus* and *P. aeruginosa*. The developed TaqMan™-OpenArray™ assay will become the method of choice for dermatophytes and nondermatophyte nail pathogens.

The high sensitivity of TaqMan™-OpenArray™ (and other PCR assays) is a key feature over standard diagnostic. Therapy becomes more efficient as molecular test results become more readily accessible. For example, alopecia in large areas could be avoided in cases of tinea capitis. Furthermore, molecular methods can detect fungal elements that are inhibited by patients self-medicating prior to seeking medical advice or during the follow-up of antimycotic therapies.

5. Conclusions

We conclude that the accuracy of the nail panel was 100% across all assays and controls tested, the assay specificity was 99.90%, overall reproducibility, which included day-to-day as well as operator-to-operator variation, was 99.91%, and the precision across all but one assay and samples resulted in standard deviations within the recommended ranges for most samples (≤1 across days and ≤0.5 within days). In addition, the assay has an excellent detection limit of 10,000-20,000 copies input to extraction, and the linear dynamic range for all assays spanned at least 5 logs with an R^2 of >0.97 for most assays. However, this test can only detect the microorganisms specified in Table 3.

6. Limitations of the Assay

Errors that may cause amplification issues include contamination of samples, sample concentration below detection level, and assay-to-assay contamination from neighboring through-holes. Errors that cause unusable amplification results include air bubbles present in the arrays after filling and sealing, PCR inhibitors, through-holes not being correctly filled with master mix or sample (plate preparation and plate loading), and OpenArray plate leak.

Data Availability

No data is left behind, and all information is shared in this manuscript.

Disclosure

This study was conducted under Suretox Laboratory LLC's in-house research and development program.

Conflicts of Interest

The authors do not have a potential conflict of interest.

Authors' Contributions

IAK and SK designed the experiments. IIA carried out the accession and extraction, while IAK performed the TaqMan™-OpenArray™ assay. IAK, SK, and SA wrote the manuscript with input from all authors.

References

[1] N. T. de Aguiar Peres, F. C. A. Maranhão, A. Rossi, and N. M. Martinez-Rossi, "Dermatophytes: host-pathogen interaction and antifungal resistance," *Anais Brasileiros de Dermatologia*, vol. 85, no. 5, pp. 657–667, 2010.

[2] M. Ragazzo, G. Puleri, V. Errichiello et al., "Evaluation of OpenArray™ as a genotyping method for forensic DNA phenotyping and human identification," *Genes*, vol. 12, no. 2, p. 221, 2021.

[3] M. Ndiaye, R. Sacheli, K. Diongue et al., "Evaluation of the multiplex real-time PCR DermaGenius((R)) assay for the detection of dermatophytes in hair samples from Senegal," *Journal of Fungi*, vol. 8, no. 1, p. 11, 2021.

[4] S. Yang and R. E. Rothman, "PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings," *The Lancet Infectious Diseases*, vol. 4, no. 6, pp. 337–348, 2004.

[5] T. Ohst, C. Kupsch, and Y. Gräser, "Detection of common dermatophytes in clinical specimens using a simple quantitative real-time TaqMan polymerase chain reaction assay," *The British Journal of Dermatology*, vol. 174, no. 3, pp. 602–609, 2016.

[6] C. Broccanello, L. Gerace, and P. Stevanato, "QuantStudio™ 12K Flex OpenArray® System as a tool for high-throughput genotyping and gene expression analysis," *Methods in Molecular Biology*, vol. 2065, pp. 199–208, 2020.

[7] E. M. Burd, "Validation of laboratory-developed molecular assays for infectious diseases," *Clinical Microbiology Reviews*, vol. 23, no. 3, pp. 550–576, 2010.