A rapid and automated sample-to-result *Candida auris* real-time PCR assay for high throughput testing of surveillance samples with BD MAX™ open system

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ABSTRACT

The multidrug-resistant yeast pathogen *Candida auris* continues to cause outbreaks and clusters of clinical cases worldwide. Previously, we developed a real-time PCR test for the detection of *C. auris* from surveillance samples (Leach et al. JCM. 2018: 56, e01223-17); the test played a crucial role in the ongoing investigations of *C. auris* outbreak in New York City. To ease the implementation of the test in other laboratories, we developed an automated sample-to-result real-time *C. auris* PCR using BD MAX™ open system, a platform proven to be adaptable for direct detection of other fungal pathogens. We optimized sample extraction at three different temperatures and four incubation periods, sensitivity was determined using eight pools of patient samples, and specificity was calculated using four clades of *C. auris*, and closely and distantly related yeasts. Three independent extractions and testing of two patient sample pools in the quadruplicate yielded assay precision. BD MAX™ optimum assay conditions were: DNA extraction at 75°C, 20 min, and the use of PerfeCTa Multiplex qPCR Toughmix. The limit of detection (LOD) of the assay was one *C. auris* CFU/PCR reaction; we detected all four clades of *C. auris* without cross-reactivity to other yeasts. Of the 110 patient surveillance samples tested, 49 were positive for *C. auris* using the BD MAX™ System with 94% clinical sensitivity and 94% accuracy compared to the manual assay. BD MAX™ assay allows high throughput screening of 180 *C. auris* surveillance samples in a 12-hour workday.

39 *Candida auris*, an emerging multidrug-resistant yeast pathogen, continues to
40 cause outbreaks and clusters of clinical cases worldwide[1, 2]. There are ongoing efforts to
41 devise better diagnostic approaches for the rapid detection of *C. auris* in clinical and
42 surveillance samples[3-5]. Previously, we developed and validated a manual real-time
43 PCR test for the detection of *C. auris* from surveillance samples at the New York State
44 Department of Health (NYSDOH) Mycology Laboratory (Leach et al. JCM. 2018: 56,
45 e01223-17). The laboratory-developed test (LDT) enabled NYSDOH laboratory scientists
46 and epidemiologists to carry out unprecedented surveillance and testing in hospitals
47 and healthcare facilities in New York City. To date, over 13,000 clinical samples from
48 151 facilities, and over 1,000 *C. auris* isolates were processed from likely the largest,
49 sustained outbreak documented globally [2]. *Candida auris* LDT, standard operating
50 procedures, and validation results were shared extensively with the hospital,
51 commercial, and public health laboratories (S. Chaturvedi, unpublished data).
52 However, the adoption of *C. auris* LDT assay is progressing slowly while the affected
53 facilities and sample numbers continue to grow. *Candida auris* real-time PCR assay was
54 not amenable to automation and high throughput screening. Therefore, we developed
55 and validated a real-time PCR for *C. auris* using BD MAX™ System, a fully integrated
56 and automated platform amenable to direct detection of other fungal pathogens[6, 7].

57 As we aimed to migrate the manual real-time PCR test to BD MAX™, the
58 primers, and probe, and PerfeCTa Multiplex qPCR ToughMix (Quanta Biosciences),

59 were evaluated as per details reported earlier along with BD MAX™ DNA MMK SPC
60 master mix[3]. We optimized BD MAX™ ExK™ DNA 1 (Plasma/Serum/Urine) DNA
61 Extraction Kit on the BD MAX™ using three different temperatures and four incubation
62 times. We carried out the entire sample-to-result procedure in the BD MAX™ PCR
63 Cartridges. BD MAX™ assay sensitivity was determined using eight pools of patient
64 samples in parallel testing with the manual real-time PCR test. We determined assay
65 specificity by the analysis of four clades of *C. auris,* and closely and distantly related
66 yeasts at high and low concentrations. The primers and probe set were previously
67 screened against a more extensive panel comprising of closely and distantly related
68 fungi, bacteria, viruses, and parasites[3]. The assay precision was determined by testing
69 samples in three independent extractions and testing two patient pools in the
70 quadruplicate. Following validation, we used the assay for the screening of 110
71 surveillance samples. GraphPad Prism 8 software (GraphPad Software, Inc., La Jolla,
72 CA) was used for statistical analysis. We used student t-test for analysis of the means
73 and considered a P value of <0.05 statistically significant. The manual *C. auris* real-time
74 PCR assay was selected as the "gold standard" to assess the diagnostic value of BD
75 MAX™ assay in *C. auris* detection from surveillance samples. BD MAX™ assay hands-
76 on time estimation involved the sum of the time required to complete sample
77 preparation, device loading, and cleaning, and result review and reporting.

78 We determined the optimum sample extraction condition to be 75°C for 20 min
79 on the BD MAX™ (Supplementary figures 1). The PerfeCTa Multiplex qPCR Toughmix
80 allowed for more efficient amplification of *C. auris* DNA than the BD MAX™ DNA
81 MMK (SPC) master mix (Supplementary figures 2). The BD MAX™ assay was highly
82 sensitive with the limit of detection (LOD) of one *C. auris* CFU/PCR reaction within Ct
83 range of 18-37 (Figure 1A). The linear regression analysis showed y = -3.66,446x + 37.49
84 and R^2= 0.9995. The assay detected all four known clades of *C. auris* with similar Ct
85 values for the same concentration of cells (Supplementary Table 1). The assay was
86 highly specific as no cross-reactivity was seen against two closely related yeasts, *C.*
87 *haemulonii* and *C. duobushaemulonii*, and 2 distantly related yeasts, *C. albicans*, and *C.*
88 *glabrata*, tested at high (1 x10^4 cells/PCR reaction) and low (1 x 10^1 cells/PCR reaction)
89 concentrations (Supplementary Table 1). The assay was highly reproducible as it
90 produced consistent Ct values for a sample pool on different days and within the same
91 day of testing (Supplementary Table 2). Of the 110 surveillance samples tested, 49 were
92 positive for *C. auris* using the BD MAX™ System, resulting in 94% clinical sensitivity
93 and 94% accuracy when compared to the manual real-time PCR assay (Table 1; Figure
94 1B). We estimated the automated sample-to-result *C. auris* BD MAX™ assay to allow
95 screening of 180 samples in a 12-hour workday.

96 We developed and validated the first automated sample-to-result real-time PCR
97 assay for high throughput testing of *C. auris*. The highlights of this study are: easy

98 migration of the manual real-time PCR assay to an automated platform, the consistent
99 performance of primers, probes, and master mix from our manual assay, comparable
100 sensitivity and specificity, and easy integration in the laboratory workflow. These
101 findings also confirm the suitability of BD MAX™ platform for direct detection of
102 fungal pathogens from clinical specimens as reported earlier for *Pneumocystic jirovecii*
103 and *Coccidioides immitis* [6,7]. Ahmad et al. (2019) partially modified our manual real-time
104 PCR assay by carrying out DNA extraction from suspected *C. auris* surveillance samples
105 on MagNA Pure 96 automated extraction system (Roche Diagnostic System,
106 Indianapolis, IN, USA); these authors reported a good correlation of the modified test
107 findings with the results obtained by cell culture and MALDI-TOF-MS identification [4].
108 The authors further claimed that partial automation of the real-time PCR assay allowed
109 testing of approximately 200 samples per day. However, the trend in infectious disease
110 diagnosis is towards full automation, multiplexing, and miniaturization of assay
111 systems to facilitate point-of-care (POC) diagnosis rapidly [8,9]. It is encouraging to note
112 that another group has successfully migrated manual real-time PCR assays to multiple
113 sample-to-results platforms such as BD MAX, ELITe InGenius (ELITechGroup), and
114 ARIES (Luminex) simultaneously[10]. In summary, the development and validation of the
115 rapid and automated sample-to-result *C. auris* BD MAX™ open system assay would
116 allow for the wider adaptability and availability of surveillance testing at the front-line
117 laboratories.

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L. Leach assisted in the design of the assay, performed the experiments and tabulated and analyzed the data. A. Russel assisted in the design of the assay, performed the experiments and participated in the analysis of data. S. Chaturvedi conceived and designed the study, interpreted data, and edited the manuscript. V. Chaturvedi, conceived and designed the study, interpreted data and wrote the manuscript. All authors commented upon the final manuscript.

131 Figure legends

132 Figure 1. BD MAX™ *Candida auris* assay sensitivity. The pool of surveillance samples

133 ranging in *C. auris* CFU from 5 x 10^{-1}-10^{5}/PCR reactions were run in duplicate on three

134 different days. The assay was linear over six orders of magnitude, and the limit of

135 detection limit was one CFU/PCR reaction on BD MAX and manual assay.

136

137 Figure 2. Correlation between *Candida auris* BD MAX™ real-time PCR and manual real-

138 time PCR. Ct distribution of 110 patient surveillance samples showed that BD MAX

139 real-time PCR assay had an excellent correlation for the detection of *C. auris* DNA with

140 manual real-time PCR assay for most of the samples (r = 0.9955).

141

142 Supplementary figures

143 Supplementary Figure 1. Comparison of efficacy of two different master mixes with

144 different extraction conditions on the BD MAX™ platform.

145

146 Supplementary Figure 2. Performance of BD MAX™ at three different temperatures and

147 four incubation periods determined with two patient pools.

148

149 Table 1. Comparison of Test Performance

150

BD MAX Real-Time PCR Result	Manual Real-Time PCR Result		Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative					
Positive	45	4	94%	94%	94%	92%	95%
Negative	3	58					

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154 Figure 1

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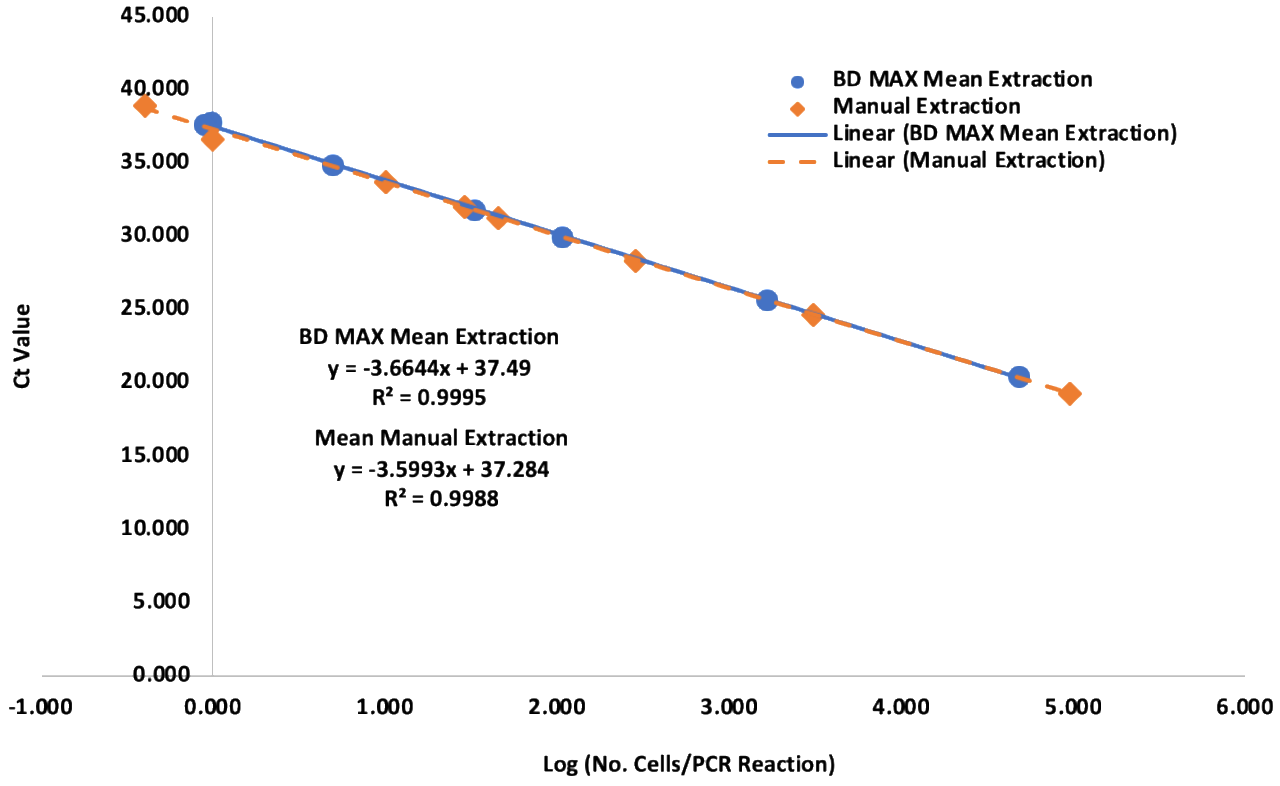

157 Figure 2

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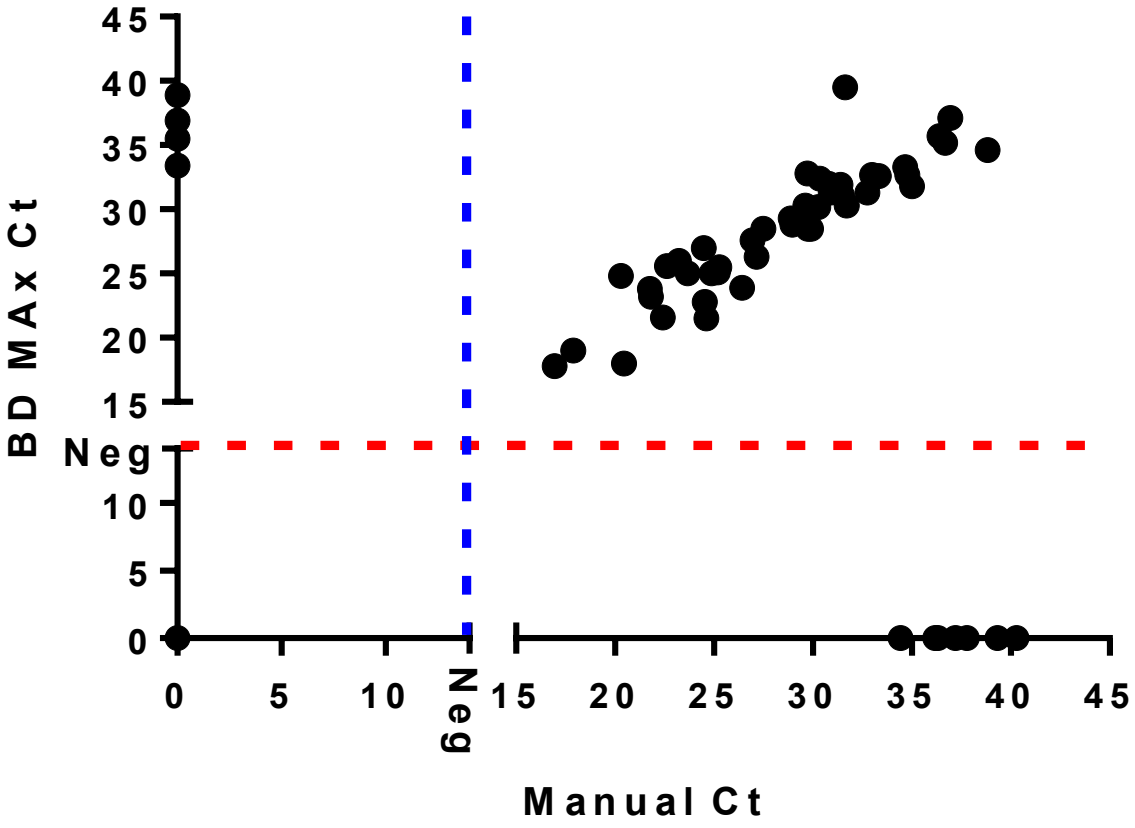

160 Supplementary Figure 1.

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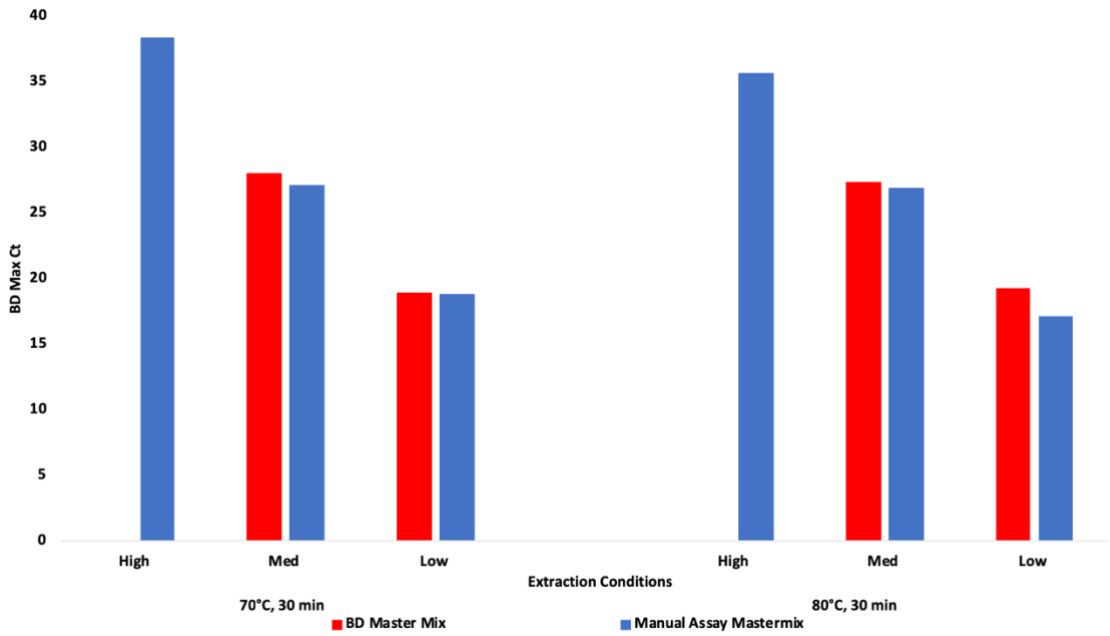
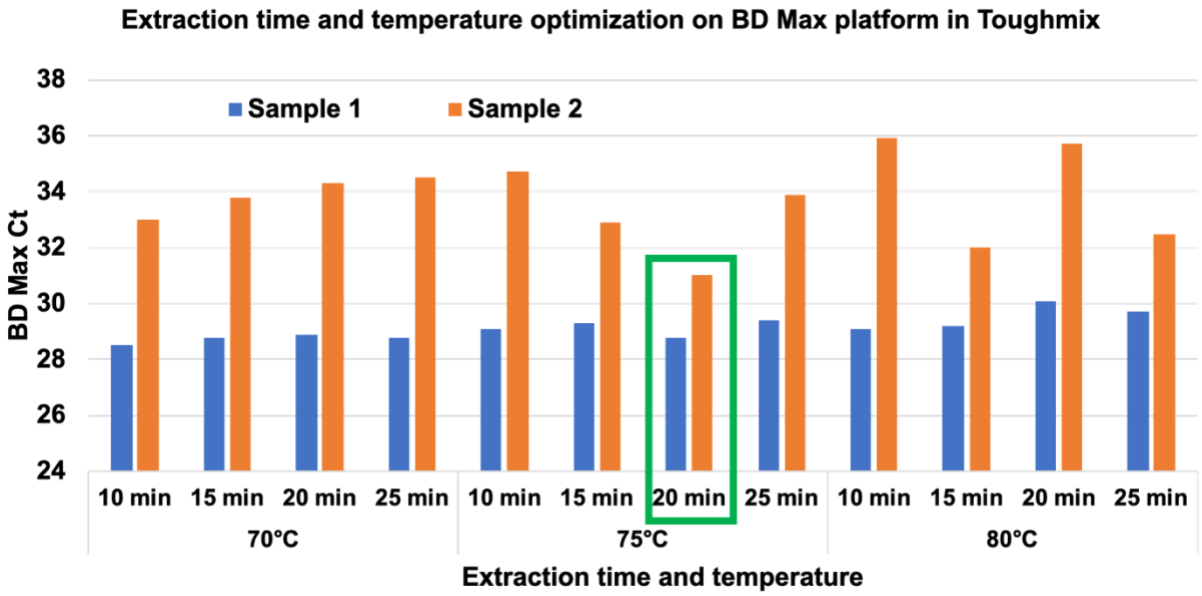


162 Supplementary Figure 2.



163 Supplementary Table 1. Specificity testing BD MAX™ real-time PCR assay

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Fungus (accession no.)*	CFU/PCR Reaction	Manual Ct	BD Max Ct
C. auris South Asia Clade (M5658)	1×10^4	21.51	23.3
	1×10^1	33.35	34.4
C. auris South Africa Clade (M5948)	1×10^4	22.45	23.5
	1×10^1	37.02	34.8
C. auris South America Clade (M5952)	1×10^4	21.17	21.3
	1×10^1	32.26	33.1
C. auris East Asia Clade (M5956)	1×10^4	17.84	19.5
	1×10^1	31.71	34.2
C. haemulonii (M8541)	1×10^4	Undet	-1
	1×10^1	Undet	-1
C. duobushaemulonii (M3051)	1×10^4	Undet	-1
	1×10^1	Undet	-1
C. albicans (M3223)	1×10^4	Undet	-1
	1×10^1	Undet	-1
C. glabrata (M8984)	1×10^4	Undet	-1
	1×10^1	Undet	-1

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166 * NYSDOH Mycology Laboratory Culture Collection Repository

167 Supplementary Table 2. Intraassay reproducibility with two pools of samples test in

168 four lanes of BD Max.

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Sample type	BD Max Ct	BD Max Ct SD	%CV
Pooled Axilla/Groin Swabs	27.1	0.48	1.79
	27.2		
	27.4		
	26.3		
Pooled Nares Swabs	22.8	0.39	1.70
	22.2		
	22.9		
	23.1		

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