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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Running Title: *Candida auris* BD MAX™ assay

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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 assay for the detection of *C. auris* from surveillance samples (Leach et al. JCM. 2018: 56, e01223-17). The assay played a crucial role in the ongoing investigations of *C. auris* outbreak in New York City. To ease the implementation of the assay in other laboratories, we developed an automated sample-to-result real-time *C. auris* PCR assay using BD MAX™ open system. 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 for 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, 50 were positive for *C. auris* using the BD MAX™ System with 96% clinical sensitivity and 94% accuracy compared to the manual assay. BD MAX™ assay allows high-throughput *C. auris* screening of 180 surveillance samples in a 12-hour workday.

39 **INTRODUCTION**

40 *Candida auris*, an emerging multidrug-resistant yeast pathogen, continues to
41 cause outbreaks and clusters of clinical cases worldwide (1, 2). There are ongoing efforts
42 to devise better diagnostic approaches for the rapid detection of *C. auris* in clinical and
43 surveillance samples (3-5). Previously, we developed and validated a manual real-time
44 PCR assay for the direct detection of *C. auris* from surveillance samples at the New York
45 State Department of Health (NYSDOH) Mycology Laboratory (3). The laboratory-
46 developed test (LDT) enabled NYSDOH laboratory scientists and epidemiologists to
47 carry out unprecedented surveillance and testing in hospitals and healthcare facilities in
48 New York City. To date, over 13,000 clinical samples from 151 healthcare facilities and
49 over 1,000 *C. auris* isolates were processed (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 *C. auris* LDT is not amenable to automation and high-throughput
53 screening, and adoption of the LDT has progressed slowly while the affected facilities
54 and sample numbers continue to grow. Therefore, we developed and validated a real-
55 time PCR assay for *C. auris* using BD MAX™ System, a fully integrated and automated
56 platform amenable to direct detection of other fungal pathogens (6, 7).

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59 **MATERIALS AND METHODS**

60 As we aimed to migrate the manual *C. auris* real-time PCR assay to BD MAX™,
61 the primers, probe, and PerfeCTa Multiplex qPCR ToughMix (Quanta Biosciences),
62 were evaluated with the BD MAX™ DNA MMK SPC master mix (3). We optimized the
63 BD MAX™ ExK™ DNA 1 (Plasma/Serum/Urine) DNA Extraction Kit on the BD MAX™
64 using three different temperatures and four incubation periods. We carried out the
65 entire sample-to-result procedure in the BD MAX™ PCR Cartridges. BD MAX™ assay
66 sensitivity was determined using eight pools of patient surveillance samples (axilla,
67 groin, axilla-groin, and nares swabs) in parallel testing with the manual real-time PCR
68 assay. Pooled samples were used to ensure sufficient volume to test identical samples in
69 triplicate using the manual and BD MAX™ assays. High, medium, and low Ct pools
70 were created by combining equal volumes from individual swab samples with similar
71 Ct values based on the manual assay. All pooled samples were then re-run with the
72 manual assay and the BD MAX™ assay to be consistent with the results. We determined
73 assay specificity by the analysis of four clades of *C. auris* and closely and distantly
74 related yeasts at high and low concentrations. The primers and probe set were
75 previously screened against a more extensive panel comprising closely and distantly
76 related fungi, bacteria, viruses, and parasites (3). The assay precision was determined
77 by testing samples in three independent extractions and testing two patient pools in
78 quadruplicate. Following validation, we used the assay for the screening of 110

79 individual patient surveillance samples. GraphPad Prism 8 software (GraphPad
80 Software, Inc., La Jolla, CA) was used for statistical analysis. We used student t-test for
81 analysis of the means and considered a *P* value of <0.05 statistically significant. The
82 manual *C. auris* real-time PCR assay was selected as the "gold standard" to assess the
83 diagnostic performance of BD MAX™ assay for the surveillance samples. BD MAX™
84 assay hands-on time estimation involved the sum of the time required to complete
85 sample preparation, device loading and cleaning, and result review and reporting.

86

87 **RESULTS**

88 The initial experiments involved optimization of DNA extraction and
89 amplification steps with the BD MAX™ system. This included initial DNA extraction at
90 three different temperatures, 70°C, 75°C, and 80°C, five different incubation times
91 ranging from 10 to 30 minutes, and amplification using two master mixes, the PerfeCTa
92 Multiplex qPCR ToughMix (manual real-time PCR assay) and BD MAX™ DNA MMK
93 SPC master mix. The ToughMix appeared better than the BD MAX™ DNA MMK SPC
94 master mix as it allowed more efficient amplification of *C. auris* DNA (Supplementary
95 Figure 1A). Next, we fine-tuned DNA extraction time and temperature using the
96 ToughMix. Our results revealed that the optimum sample DNA extraction condition
97 was 75°C for 20 min on the BD MAX™ (Supplementary Figure 1B). The selected

98 extraction condition was further evaluated using two pooled surveillance samples
99 (axilla, groin, axilla-groin, and nares) with high and medium Ct values in quadruplicate
100 and results showed excellent reproducibility (Supplementary Table 1). In expanded
101 testing, BD MAX™ assay was highly sensitive with the limit of detection (LOD) of one
102 *C. auris* CFU/PCR reaction (Figure 1). The assay detected all four known clades of *C.*
103 *auris* with similar Ct values for the same concentration of cells (Supplementary Table 2).
104 The assay was highly specific as no cross-reactivity was seen against two closely related
105 yeasts, *C. haemulonii* and *C. duobushaemulonii*, and 2 distantly related yeasts, *C. albicans*
106 and *C. glabrata*, tested at high (1×10^4 cells/PCR reaction) and low (1×10^1 cells/PCR
107 reaction) concentrations (Supplementary Table 2). The assay was highly reproducible as
108 it produced consistent Ct values on three different days of testing (Supplementary Table
109 3A), and within the same day of testing (Supplementary Table 3B). Upon completion of
110 the test validation, we tested 110 individual patient surveillance samples; 50 were
111 positive for *C. auris* by BD MAX™ System, resulting in 96% clinical sensitivity and 94%
112 accuracy when compared to the manual real-time PCR assay (Table 1). The Ct value
113 distributions of 110 individual patient surveillance samples showed excellent
114 correlation between two methods (Figure 2). We estimated the automated sample-to-
115 result *C. auris* BD MAX™ assay to allow the screening of 180 samples in a 12-hour
116 workday.

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118 **DISCUSSION**

119 We developed and validated the first automated sample-to-result real-time PCR
120 assay for high-throughput testing of *C. auris*. The highlights of this study are: easy
121 migration of the manual real-time PCR assay to an automated platform, the consistent
122 performance of primers, probe, and master mix from our manual assay, comparable
123 accuracy, sensitivity and specificity, and easy integration in the laboratory workflow.
124 These findings also confirm the suitability of BD MAX™ platform for direct detection of
125 fungal pathogens from clinical specimens as reported earlier for *Pneumocystis jirovecii*
126 and *Coccidioides immitis* (6, 7). There was indication that BD MAX™ performed better
127 than the manual assay for a few samples with high Ct values possibly due to the
128 elimination of many hands-on steps and the use of purified DNA. Although the
129 samples tested in the present study belonged to *C. auris* South Asia clade I, our primers
130 and probe have performed equally well with other well-known *C. auris* clades in the
131 validation panel and the manual assay described earlier (3). Ahmad et al. (2019)
132 partially modified our manual real-time PCR assay by carrying out DNA extraction
133 from suspected *C. auris* surveillance samples on MagNA Pure 96 automated extraction
134 system (Roche Diagnostic System, Indianapolis, IN, USA); these authors reported a
135 good correlation of the modified test findings with the results obtained by cell culture
136 and MALDI-TOF-MS identification (4). The authors further claimed that partial
137 automation of the real-time PCR assay allowed testing of approximately 200 samples

138 per day. However, the trend in infectious disease diagnosis is towards full automation,
139 multiplexing, and miniaturization of assay systems to facilitate rapid point-of-care
140 (POC) diagnosis (8, 9). It is encouraging to note that another group has successfully
141 migrated manual real-time PCR assays to multiple sample-to-result platforms such as
142 BD MAX™, ELITe InGenius (ELITechGroup), and ARIES (Luminex)
143 simultaneously(10). In summary, the development and validation of the rapid and
144 automated sample-to-result *C. auris* BD MAX™ open system assay would allow for the
145 wider adaptability and availability of surveillance testing at the front-line laboratories.
146

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152 L. Leach assisted in the assay's design, performed the experiments, and tabulated and
153 analyzed the data. A. Russell assisted in the assay's design, performed the experiments,
154 and participated in the analysis of data, and edited the manuscript. Y. Zhu performed
155 experiments and tabulated and analyzed the data and edited the manuscript. S.
156 Chaturvedi conceived and designed the study, interpreted data, and edited the

157 manuscript. V. Chaturvedi conceived and designed the study, interpreted data and

158 wrote the manuscript. All authors commented upon the final manuscript.

159

160 **FIGURE LEGENDS**

161 Figure 1. BD MAX™ *Candida auris* assay sensitivity. The pool of patient surveillance

162 samples ranging in *C. auris* CFU from 5×10^{-1} - 5×10^{5}/PCR reactions were run in

163 duplicate on three different days. The assay was linear over six orders of magnitude,

164 and the limit of detection limit was one CFU/PCR reaction on BD MAX™ and manual

165 assay.

166

167 Figure 2. Side-by-side comparison between *Candida auris* BD MAX™ real-time PCR and

168 manual real-time PCR assays. Ct distribution of 110 individual patient surveillance

169 samples showed that BD MAX™ real-time PCR assay had an excellent correlation for

170 the detection of *C. auris* DNA with manual real-time PCR assay for most of the samples

171 (r = 0.9955).

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175 Table 1. Comparison of Test Performance of Individual Patient Surveillance Sample

BD MAX™ Real-Time PCR Result	Manual Real-Time PCR Result		Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative					
Positive	45	5	94%	96%	92%	92%	97%
Negative	2	58					

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180 References

181 1. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, Colombo AL,
182 Calvo B, Cuomo CA, Desjardins CA. 2017. Simultaneous Emergence of Multidrug-Resistant
183 *Candida auris* on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological
184 Analyses. Clinical Infectious Diseases 64:134-140.
185 2. Adams E, Quinn M, Tsay S, Poirot E, Chaturvedi S, Southwick K, Greenko J, Fernandez R, Kallen A,
186 Vallabhaneni S, Haley V, Hutton B, Blog D, Lutterloh E, Zucker H, Workgroup. *Ca*l. 2018. *Candida*
187 *auris* in Healthcare Facilities, New York, USA, 2013-2017. Emerging Infectious Diseases 24:1816-
188 1824.
189 3. Leach L, Zhu Y, Chaturvedi S. 2018. Development and validation of a real-time PCR assay for
190 rapid detection of *Candida auris* from surveillance samples. Journal of Clinical Microbiology
191 56:e01223-17.
192 4. Ahmad A, Spencer JE, Lockhart SR, Singleton S, Petway DJ, Bagarozzi DA, Jr., Herzegh OT. 2019. A
193 high-throughput and rapidm ethod for accurate identification of emerging multidrug-resistant
194 *Candida auris*. Mycoses 62:513-518.
195 5. Arastehfar A, Fang W, Daneshnia F, Al-Hatmi AM, Liao W, Pan W, Khan Z, Ahmad S, Rosam K,
196 Lackner M, Lass-Florl C, Hagen F, Boekhout T. 2019. Novel multiplex real-time quantitative PCR
197 detecting system approach for direct detection of *Candida auris* and its relatives in spiked serum
198 samples. Future Microbiol 14:33-45.
199 6. Dalpke AH, Hofko M, Zimmermann S. 2013. Development and evaluation of a real-time PCR
200 assay for detection of *Pneumocystis jirovecii* on the fully automated BD MAX platform. Journal
201 of Clinical Microbiology 51:2337-2343.
202 7. Mitchell M, Dizon D, Libke R, Peterson M, Slater D, Dhillon A. 2015. Development of a real-time
203 PCR assay for identification of *Coccidioides immitis* by use of the BD Max system. Journal of
204 Clinical Microbiology 53:926-929.
205 8. Dincer C, Bruch R, Kling A, Dittrich PS, Urban GA. 2017. Multiplexed point-of-care testing –
206 xPOCT. Trends in Biotechnology 35:728-742.
207 9. Zarei M. 2018. Infectious pathogens meet point-of-care diagnostics. Biosensors and
208 Bioelectronics 106:193-203.
209 10. Frans G, Beuselinck K, Peeters B, Van Ranst M, Saegeman V, Desmet S, Lagrou K. 2019. Migrating
210 a lab-developed MERS-CoV real-time PCR to 3 “Sample to Result” systems: experiences on
211 optimization and validation. Diagnostic Microbiology and Infectious Disease
212 doi:https://doi.org/10.1016/j.diagmicrobio.2019.02.006.

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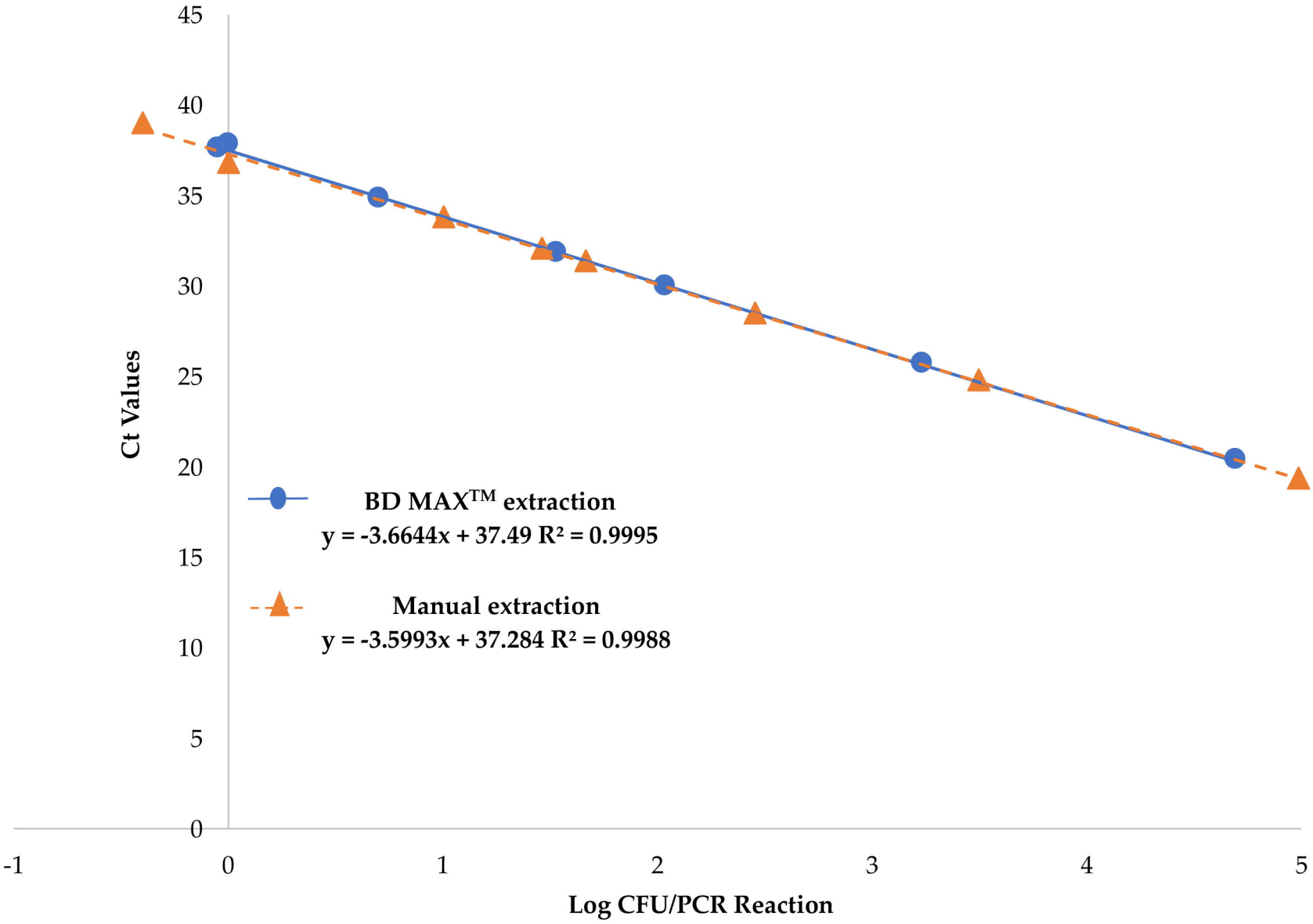
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Ct Values
-1
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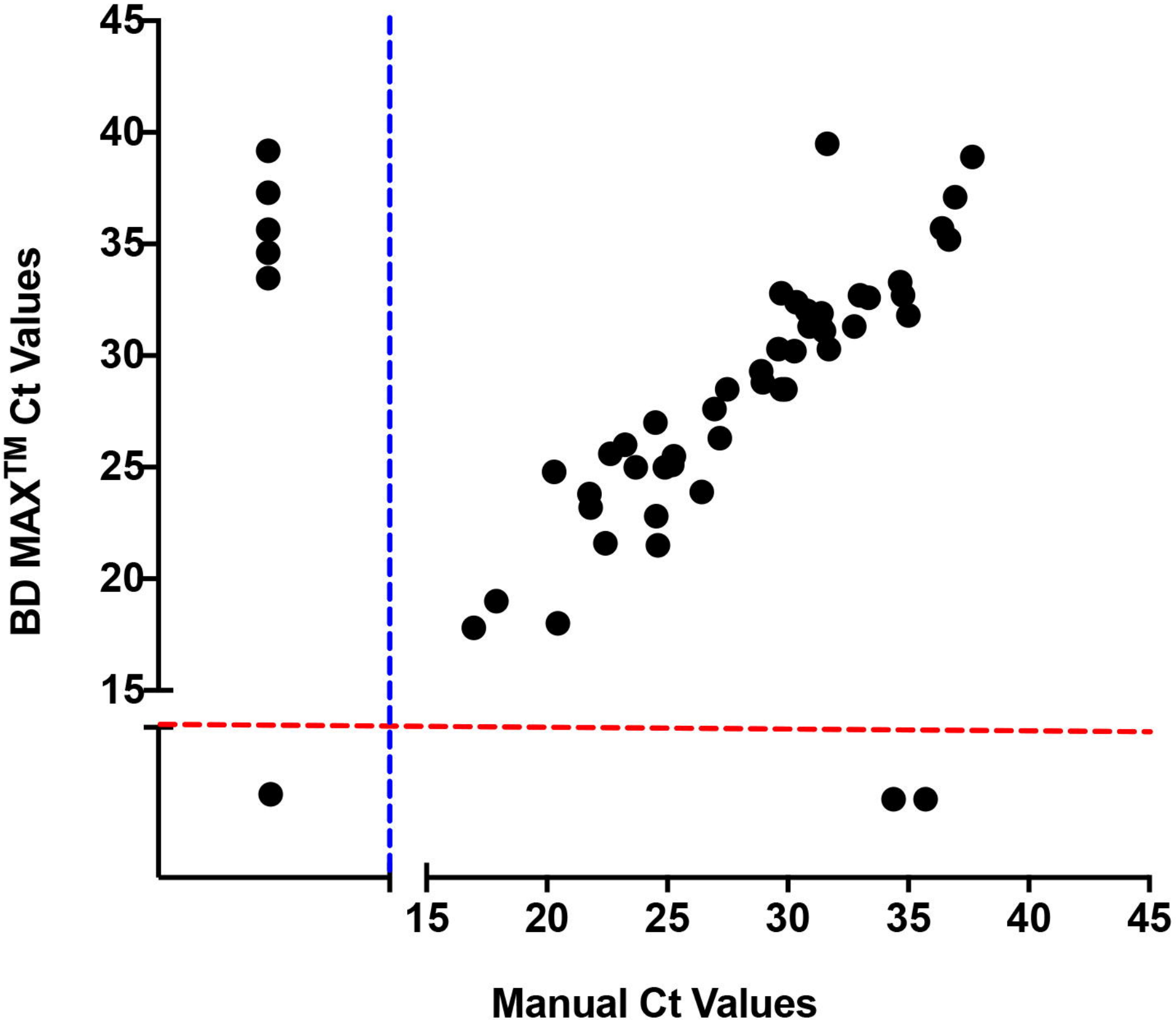
Log CFU/PCR Reaction
BD MAX™ extraction
y = -3.6644x + 37.49 R² = 0.9995
Manual extraction
y = -3.5993x + 37.284 R² = 0.9988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BD MAX™ Ct Values
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Manual Ct Values