

1 **Comparison of Cepheid Xpert Xpress and Abbott ID Now to Roche cobas for the Rapid**
2 **Detection of SARS-CoV-2**

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27 **Abstract:**

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29 The SARS-CoV-2 pandemic has created an urgent and unprecedented need for rapid
30 large-scale diagnostic testing to inform timely patient management. This study compared two
31 recently-authorized rapid tests, Cepheid Xpert Xpress SARS-CoV-2 and Abbott ID Now SARS-
32 CoV-2 to the Roche cobas SARS-CoV-2 assay. A total of 113 nasopharyngeal swabs were
33 tested, including 88 positives spanning the full range of observed C_t values on the cobas assay.
34 Compared to cobas, the overall positive agreement was 73.9% with ID Now and 98.9% with
35 Xpert. Negative agreement was 100% and 92.0% for ID Now and Xpert, respectively. Both ID
36 Now and Xpert showed 100% positive agreement for medium and high viral concentrations (C_t
37 value <30). However, for C_t values >30, positive agreement was 34.3% for ID Now and 97.1%
38 for Xpert. These findings highlight an important limitation of ID Now for specimens collected in
39 viral or universal transport media with low viral concentrations. Further studies are needed to
40 evaluate the performance of ID Now for direct swabs.

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50 **Introduction:**

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52 Severe acute respiratory virus coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in
53 December 2019 and has since rapidly spread across the world, causing a global pandemic of
54 coronavirus disease (COVID-19). The majority of cases are mild to moderate, but severe
55 infections have overwhelmed healthcare systems in the United States, particularly in New York
56 City. Real-time polymerase chain reaction (RT-PCR) of viral RNA from nasal or nasopharyngeal
57 swabs has become the standard method used to confirm diagnosis. The first quantitative RT-PCR
58 test for detecting SARS-CoV2 was designed and distributed in January 2020 by the World
59 Health Organization (WHO) (1). In the United States and many other countries, however, the
60 slow rollout of large-scale diagnostic testing and the long turnaround times associated with
61 laboratory tests, particularly those sent to reference laboratories, have significantly hampered
62 public health efforts to contain the outbreak.

63 In contrast, commercially-available rapid diagnostic assays can better inform timely
64 patient management decisions to guide the need for quarantine, isolation, contact tracing, and
65 therapeutic management. Beginning in March 2020, multiple SARS-CoV-2 rapid tests received
66 Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA).
67 However, manufacturer submissions for EUA only require evaluation of the limit of detection
68 and cross-reactivity of their assays, and do not address other important performance
69 characteristics such as accuracy, precision, and reproducibility. In addition, most manufacturer
70 submissions include studies of contrived positive samples with spiked viral RNA, and do not
71 assess performance on clinical patient specimens. Two recently-authorized rapid tests, Xpert
72 Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) and ID Now SARS-CoV-2 (Abbott, Chicago,

73 IL) have been manufactured at wide-scale and distributed to numerous medical centers around
74 the country. While limited studies on these two assays have been recently published, the number
75 of patient samples evaluated to date has been relatively small, and significant questions remain
76 about the accuracy of these tests across the full spectrum of viral loads (2-4). Utilizing the high
77 volume of patient testing performed at our medical center in New York City, we sought to
78 evaluate and compare the performance of these two rapid assays across a range of clinical
79 samples.

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81 **Materials and Methods:**

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83 *Study Design and Data Analysis:*

84 Deidentified remnant patient samples that underwent routine clinical testing with the
85 cobas SARS-CoV-2 assay on the 6800 platform (Roche Diagnostics, Indianapolis, IN) were used
86 to evaluate the Xpert and ID Now assays. Residual nasopharyngeal (NP) swabs in transport
87 media were held at 4° C prior to testing on the Xpert and ID NOW platforms, with all testing
88 completed within 48 hours of sample collection. Testing was performed according to the
89 manufacturers' instructions on two separate ID Now instruments and a single GeneXpert Infinity
90 instrument.

91 A total of 113 NP swabs collected in 3 mL of viral transport media (M4RT VTM;
92 ThermoFisher Scientific, Waltham, MA) or universal transport media (UTM; Becton Dickinson
93 and Co., Franklin Lakes, NJ) were included.. The specimens were collected from April 8 to April
94 13, 2020 and included 111 adult and 2 pediatric patients who were all seen in inpatient or
95 emergency room locations.

96 To evaluate assay performance at varying viral concentrations, 88 positive specimens
97 were selected to represent the full range of observed C_t values on the cobas assay, ranging from
98 14 – 38 cycles. Positive agreement and 95% confidence intervals for the Xpert and ID Now
99 assays were calculated using cobas as the reference test. An additional 25 negative specimens
100 were selected to evaluate negative agreement.

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102 *Assay Descriptions:*

103 The cobas assay utilizes RT-PCR to amplify and detect two viral targets: ORF1 a/b, a
104 non-structural region that is unique to SARS-CoV-2 and a conserved region in the E-gene, which
105 is a structural protein envelope for pan-Sarbecovirus detection. The Xpert assay is an automated
106 RT-PCR that amplifies and detects two viral targets: N2, a nucleocapsid recombinant protein
107 unique to SARS-CoV-2 and a region in the structural envelope E-gene. The ID Now assay uses
108 proprietary isothermal nucleic acid amplification technology for qualitative detection of SARS-
109 CoV-2 RdRp gene using fluorescent reporter probes.

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111 This study was approved by the Columbia University Irving Medical Center Institutional Review
112 Board.

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114 **Results:**

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116 Of the 113 patient specimens, 111 were from adults ranging in age from 23 to 101 years
117 and two were from pediatric patients, aged 1 day and 5 days old. The average age was 65 years

118 for positive samples and 43 years for negative samples. Overall, the majority of positive samples
119 were from males (60.2%) and negative samples from females (68.0%) (Table 1).

120 Testing results by Abbott ID Now and Cepheid Xpert are shown in Table 2 and Figure 1.
121 Compared to cobas, the overall positive agreement with ID Now was 73.9% (95% Confidence
122 Interval (CI): 63.2 – 82.3%) and with Xpert was 98.9% (95% CI 92.9 – 100%). Negative
123 agreement was 100% (95% CI 83.4 – 100%) and 92.0% (95% CI 72.4 – 98.6%) for ID Now and
124 Xpert, respectively. Both ID Now and Xpert showed 100% positive agreement for medium and
125 high viral concentrations, defined as C_t value <30. However, for C_t values >30, positive
126 agreement for ID Now was 34.3% (95% CI 19.7 – 52.2%), whereas it was 97.1% (95% CI 83.4 –
127 99.8%) for Xpert. Notably, one sample detected by Xpert was a presumptive positive based on
128 detection of the E-gene target but not the N2 target. There were also two samples that tested
129 negative by cobas but positive by the Xpert. These samples had C_t values >40 for the N2 target
130 only without detection of the E-gene target. For the E-gene target, C_t values were generally 1
131 cycle lower for Xpert than cobas (Supplemental Materials, Table S1 and Figure S1).

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133 **Discussion:**

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135 To meet the urgent need for wide-scale diagnostic testing during the COVID-19
136 pandemic, multiple rapid molecular tests have recently been authorized by the US FDA, some of
137 which are available in point-of-care (POC) or near patient settings. However, very few studies
138 have been published to date on the relative performance characteristics of these assays,
139 especially for patient specimens representing a wide range of viral concentrations (2, 3).

140 In this comparative analysis, the Xpert assay showed a very high level of agreement with
141 the cobas assay across the entire range of tested C_t values, including low-level positives. These
142 findings confirm those published by Moran *et al.* (2) and show a high level of agreement
143 between these two assays using an expanded number of positive clinical samples. In contrast, the
144 ID Now assay reliably detected specimens with C_t values ≤ 30 , but did not detect the majority of
145 specimens with C_t values ≥ 30 . Whereas Rhoades *et al.* (3) found an overall high level of
146 agreement between ID Now and the modified CDC assay, our findings highlight an important
147 limitation of the ID Now for low-level positives. While both studies evaluated nasopharyngeal
148 swabs eluted in transport media, it is important to note that the EUA for ID NOW was recently
149 updated to remove the indication for swabs in transport media (5). Our data support that the EUA
150 was appropriately modified, as samples may become too dilute in VTM and low-level positives
151 may falsely test negative.

152 In contrast to batch testing and the higher complexity required for the cobas assay, both
153 Xpert and ID Now offer shorter turnaround times and availability in near-patient settings.
154 However, the two assays differ in throughput capacity and run time. Each ID Now platform can
155 run only a single specimen at a time, with results available in 13 minutes or less. Xpert can be
156 run on larger, random-access platforms that allow for significantly higher throughput, with
157 results available in 45 minutes. Both assays are available for use in POC settings, which
158 introduces both benefits and drawbacks. On the one hand, POC molecular testing delivers the
159 shortest possible interval between sample collection and result, which can facilitate faster clinical
160 decision-making. However, concerns related to assay performance, quality management, and
161 safety in the POC setting still remain. Studies of POC molecular testing for influenza and
162 respiratory syncytial virus have shown promising results, but also highlight some of these

163 concerns (6-9). The risk of contamination and false positives is also much higher when testing is
164 performed outside of a controlled environment and by non-laboratory trained personnel.
165 Nevertheless, the unique circumstances of a rapidly-spreading pandemic may ultimately
166 necessitate the widescale adoption of POC assays in completely new patient settings, such as
167 walk up or drive-thru testing centers.

168 Limitations of this study include the relatively few number of samples from pediatric
169 patients, as only two samples from patients aged 1 day and 5 days were included, and both of
170 these were negative on all three testing methods. We were also only able to evaluate ID Now for
171 specimens in transport media. The performance of this assay with direct nasal swabs requires
172 further evaluation in subsequent studies. Another limitation is the use of the cobas assay as the
173 comparator assay. Two samples that were identified as positive only by Xpert on the basis of N2
174 nucleocapsid gene detection were negative for both targets on cobas. Whether these samples
175 were truly positive or truly negative could not be determined.

176 Fast, readily available, and reliable test results are critically important during this
177 pandemic, and each of the three assays evaluated in this study holds promise to deliver valuable
178 clinical information. Further head-to-head comparisons of molecular tests will be important in
179 order to establish the usefulness of each method and to help medical providers determine the
180 most appropriate diagnostic tests to best serve their communities.

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213 **Figure Legends:**

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215 Figure 1A: Frequency distribution of cycle threshold (C_t) values for positive patient samples by
216 Roche cobas SARS-CoV-2 (blue), Cepheid Xpert Xpress SARS-CoV-2 (orange) and Abbott ID
217 Now SARS-CoV-2(gray) assays. Roche cobas Target 1 (ORF1) C_t values were rounded to the
218 nearest whole number.

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221 Figure 1B: Frequency distribution of cycle threshold (C_t) values for positive patient samples by
222 Roche cobas SARS-CoV-2 (blue), Cepheid Xpert Xpress SARS-CoV-2 (orange) and Abbott ID
223 Now SARS-CoV-2(gray) assays. Roche cobas Target 1 (ORF1) C_t values were rounded to the
224 nearest whole number.

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Table 1: Demographics of included patients

Cobas C_t Category	Average Age (years)	Male (%)	Female (%)
Total Positive	64.9	53 (60.2)	35 (39.8)
Low (>30)	60.9	19 (54.3)	16 (45.7)
Medium (20-30)	67.1	24 (63.2)	14 (36.8)
High (<20)	68.4	10 (66.6)	5 (33.3)
Negative	42.6	8 (32.0)	17 (68.0)

Table 2: Positive and negative agreement of Abbott ID Now SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 with Roche cobas SARS-CoV-2

Cobas C_i Category	ID Now (% , 95% CI)	Xpert (% , 95% CI)	Total
Total Positive	65 (73.9, 63.2-82.3)	87 (98.9, 92.9-99.9)	88
Low (>30)	12 (34.3, 19.7-52.2)	34* (97.1, 83.4-99.8)	35
Medium (20-30)	38 (100, 88.6-100)	38 (100, 88.6-100)	38
High (<20)	15 (100, 74.7-100)	15 (100, 74.7-100)	15
Negative	25 (100, 83.4-100)	23 (92.0, 72.4-98.6)	25

* One specimen was presumptively positive by Xpert

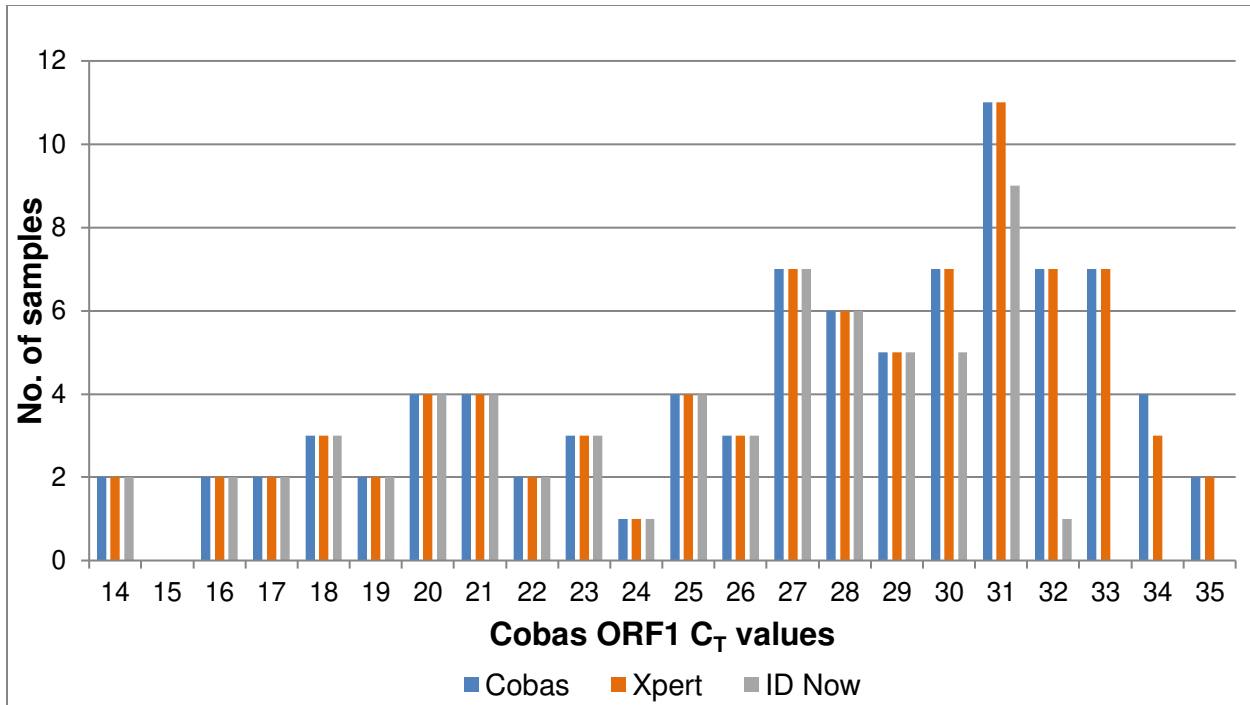


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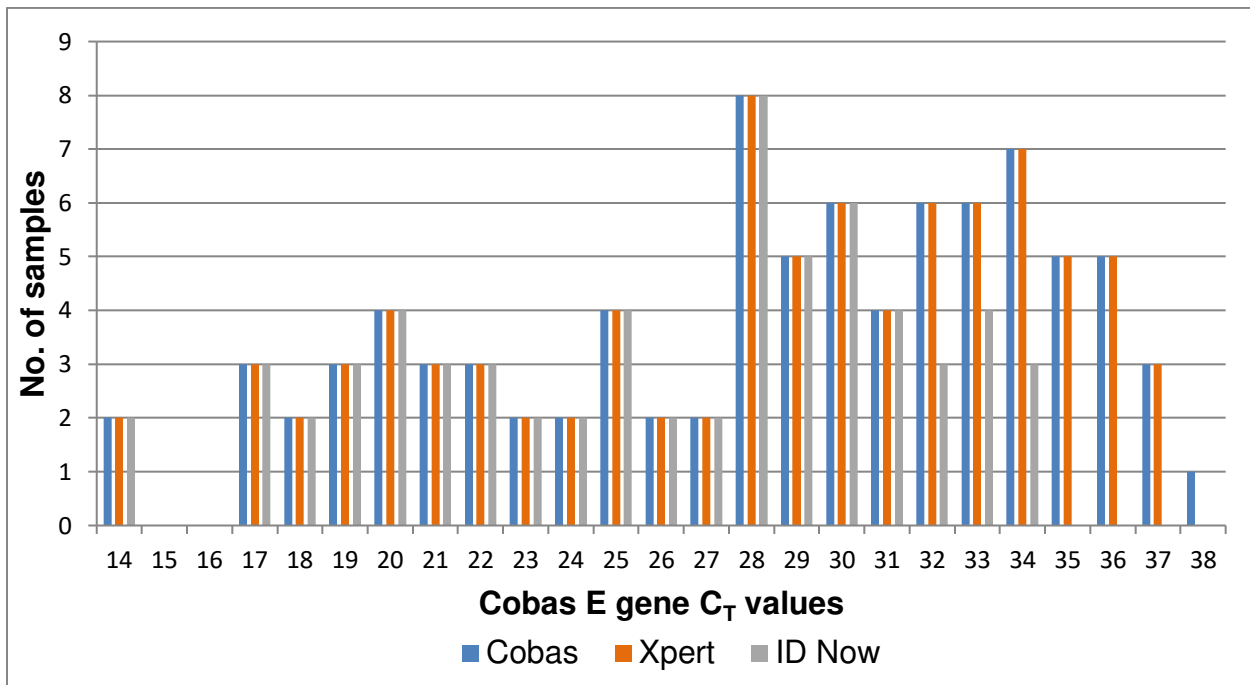


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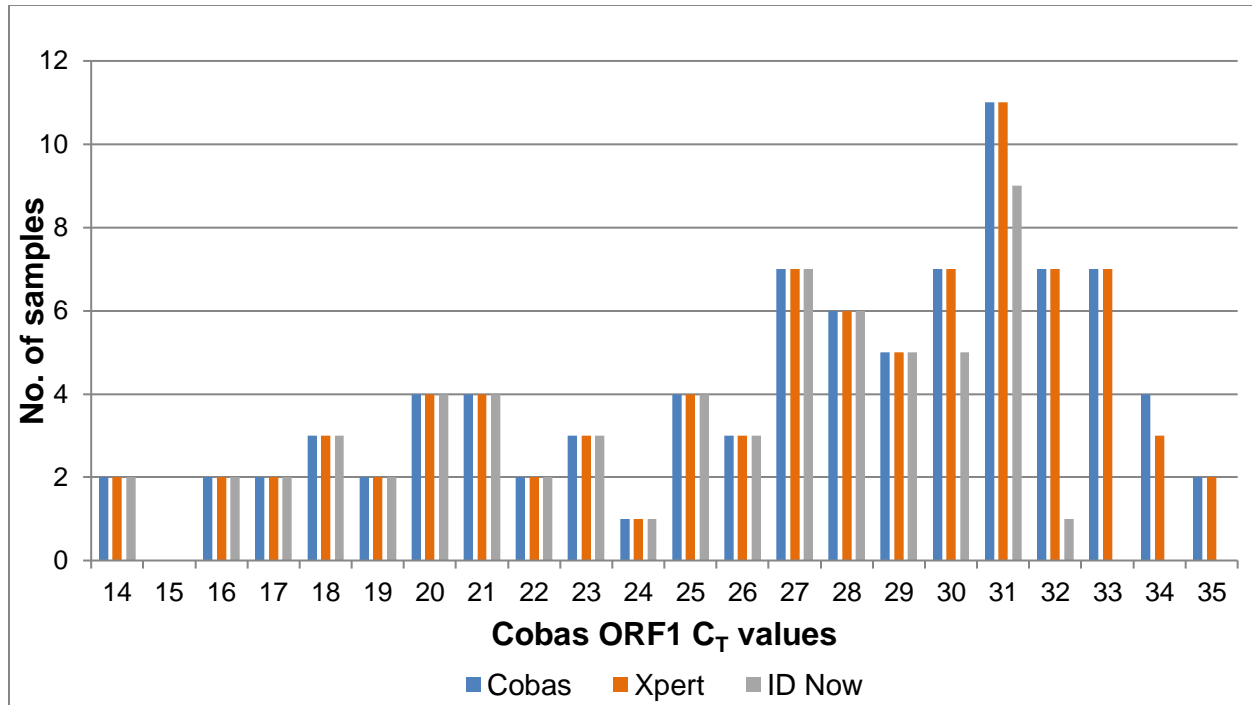


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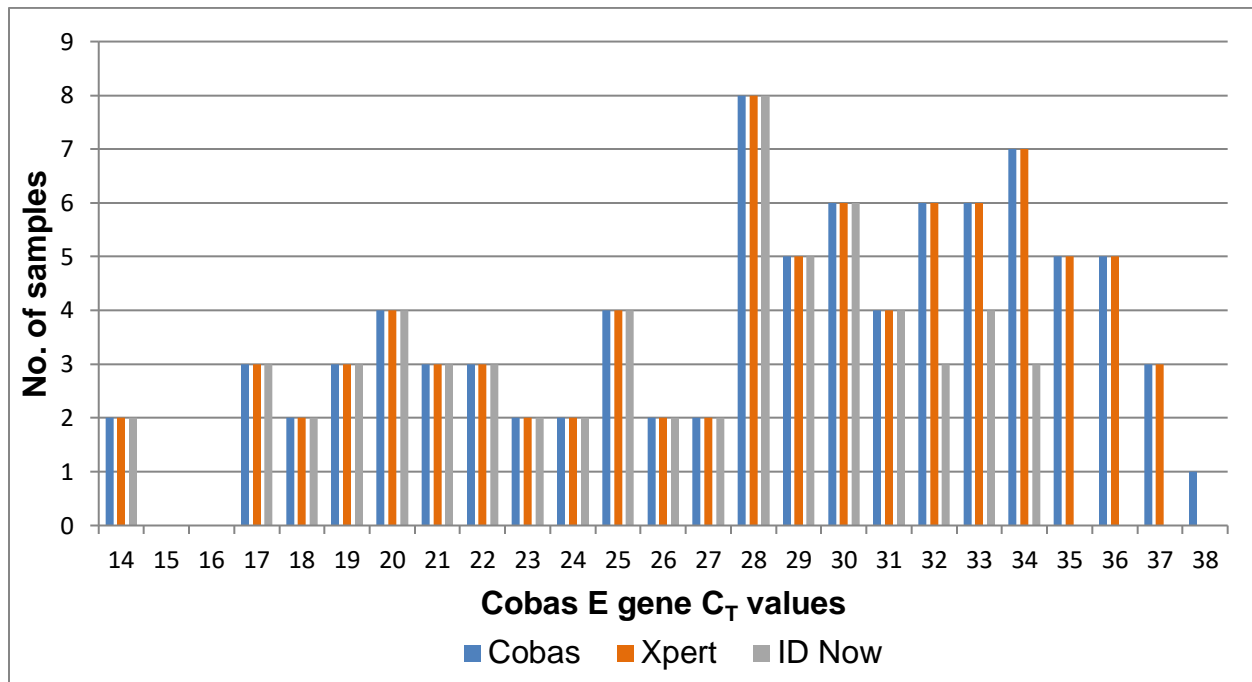


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