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

Marie C. Smithgall[1], Ioana Scherberkova[2], Susan Whittier[1], Daniel A. Green[1,#]

[1]Department of Pathology & Cell Biology, Columbia University Irving Medical Center, New York, NY

[2]Vagelos College of Physicians and Surgeons, Columbia University Irving Medical Center, New York, NY

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#Address correspondence to:

Daniel A. Green, M.D.

3959 Broadway

Microbiology, CHONY C-324

New York, NY 10032

dag2149@cumc.columbia.edu

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Abstract:

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

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.

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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

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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).

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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

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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   <u>Figure 1A:</u> 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

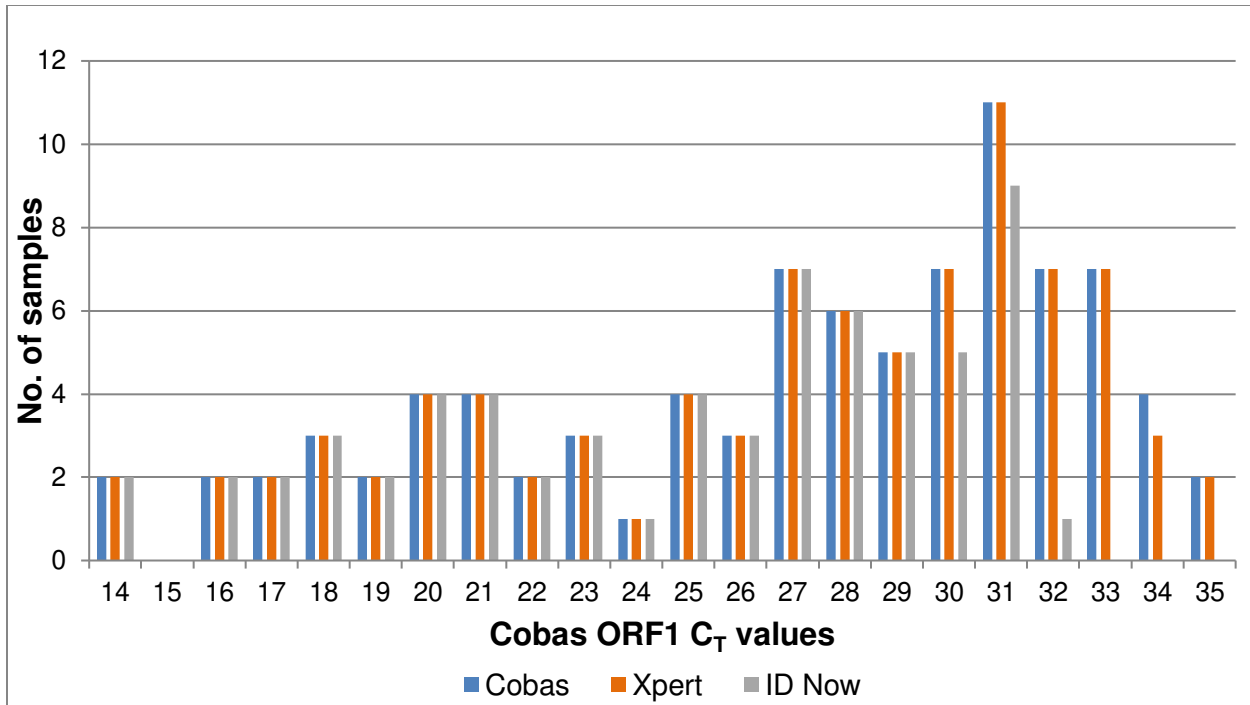218   nearest whole number.

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221   <u>Figure 1B:</u> 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_t 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

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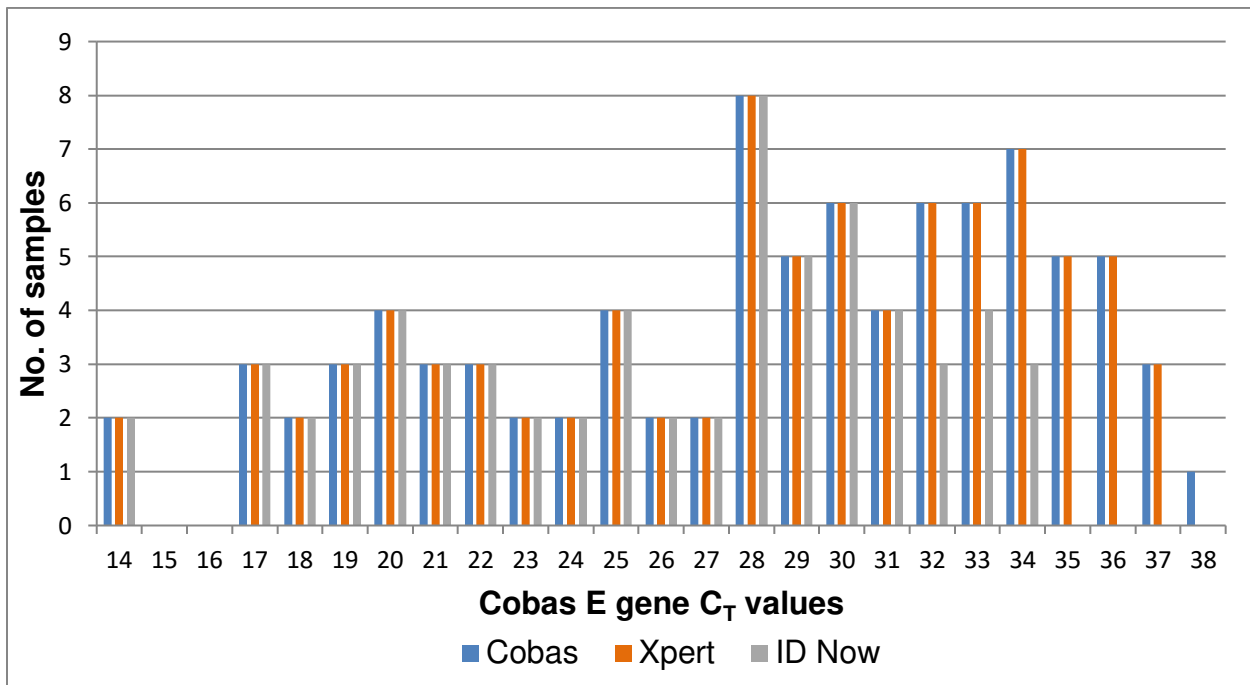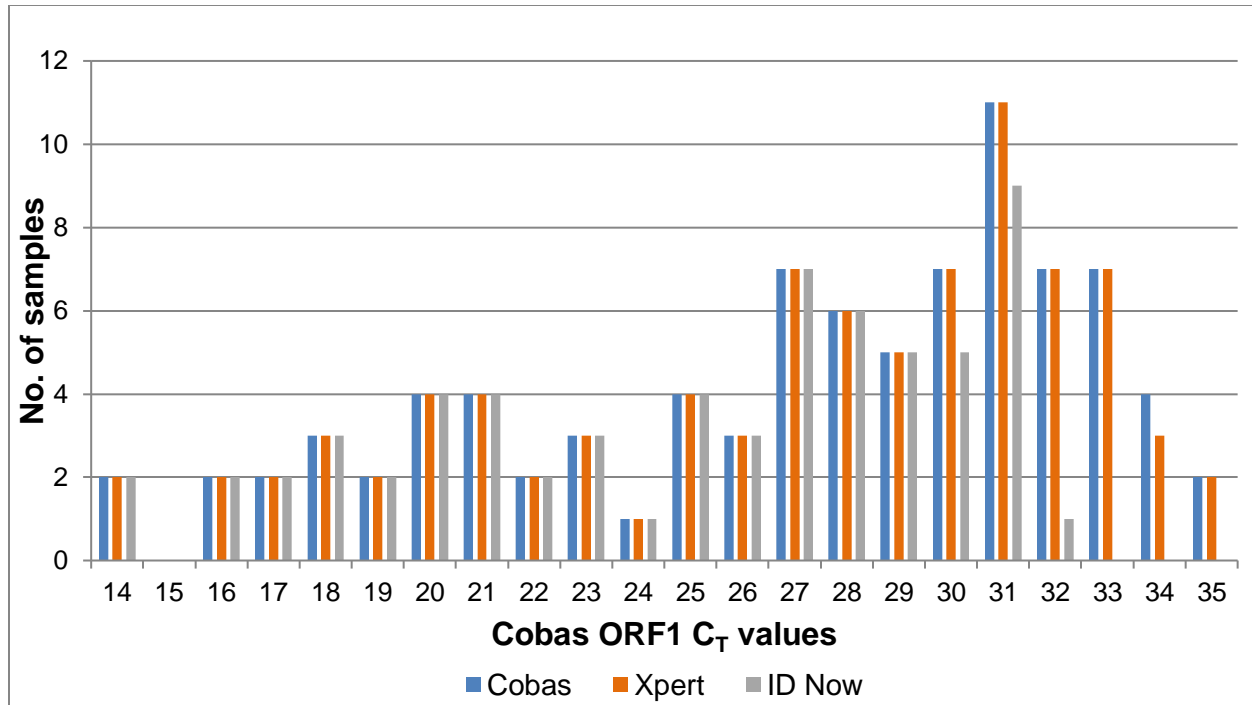

Figure 1B: Frequency distribution of cycle threshold (C_t) values for positive patient samples by Roche cobas SARS-CoV-2 (blue), Cepheid Xpert Xpress SARS-CoV-2 (orange) and Abbott ID Now SARS-CoV-2(gray) assays. Roche cobas Target 1 (ORF1) C_t values were rounded to the nearest whole number.

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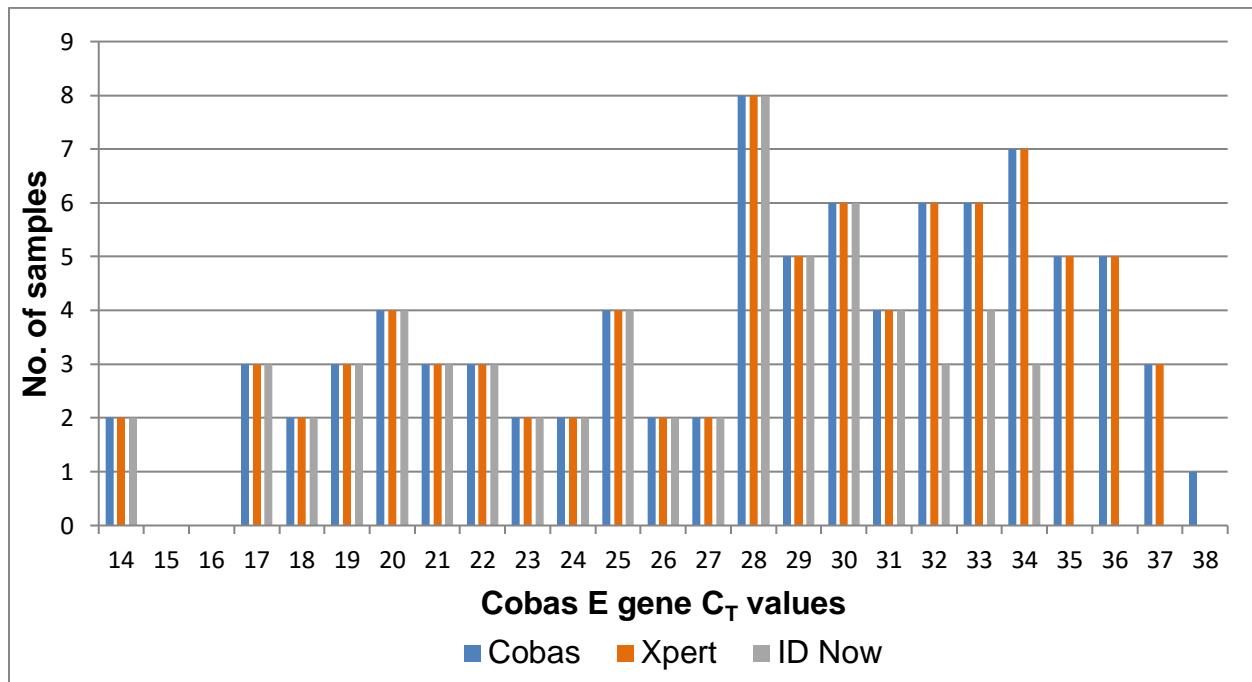

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