

# Comparison of three TaqMan Real-Time Reverse Transcription-PCR assays in detecting SARS-CoV-2

Running title: Comparison of 3 qRT-PCR assays detecting SARS-CoV-2

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## 34 Abstract

35 Quick and accurate detection of SARS-CoV-2 is critical for COVID-19 control.  
 36 Dozens of real-time reverse transcription PCR (qRT-PCR) assays have been  
 37 developed to meet the urgent need of COVID-19 control. However, methodological  
 38 comparisons among the developed qRT-PCR assays are limited. In the present study,  
 39 we evaluated the sensitivity, specificity, amplification efficiency, and linear detection  
 40 ranges of three qRT-PCR assays, including the assays developed by our group  
 41 (IPBCAMS), and the assays recommended by WHO and China CDC (CCDC). The  
 42 three qRT-PCR assays exhibited similar sensitivities, with the limit of detection (LOD)  
 43 at about 10 copies per reaction (except the ORF 1b gene assay in CCDC assays with a  
 44 LOD at about 100 copies per reaction). No cross reaction with other respiratory  
 45 viruses were observed in all of the three qRT-PCR assays. Wide linear detection  
 46 ranges from  $10^6$  to  $10^1$  copies per reaction and acceptable reproducibility were  
 47 obtained. By using 25 clinical specimens, the N gene assay of IPBCAMS assays and  
 48 CCDC assays performed better (with detection rates of 92% and 100%, respectively)  
 49 than that of the WHO assays (with a detection rate of 60%), and the ORF 1b gene  
 50 assay in IPBCAMS assays performed better (with a detection rate of 64%) than those  
 51 of the WHO assays and the CCDC assays (with detection rates of 48% and 20%,  
 52 respectively). In conclusion, the N gene assays of CCDC assays and IPBCAMS  
 53 assays and the ORF 1b gene assay of IPBCAMS assays were recommended for  
 54 qRT-PCR screening of SARS-CoV-2.

55 **Key words:** SARS-CoV-2; qRT-PCR; methodological evaluation; Limit of Detection;  
 56 reproductivity, clinical performance

## 57 **Introduction**

58 Since the first detection in late 2019, severe respiratory syndrome CoV-2  
59 (SARS-CoV-2) caused Corona Virus Infectious Disease in 2019 (COVID-19) has  
60 widely spread in the world. By April 11, 2020, more than 1.7 million patients infected  
61 by SARS-CoV-2 has been reported from 185 countries (1). Given the quick increase  
62 in confirmed cases and asymptomatic infections, there are increasing demands in  
63 diagnostic tools for quick and accurate detection of the virus (2, 3). Several real-time  
64 reverse transcription-Polymerase Chain Reaction (qRT-PCR) for the detection of  
65 SARS-COV-2 has been developed to meet the demands, including the assays by this  
66 group (IPBCAMS assays), and the assays by WHO (WHO assays), and the assays by  
67 China CDC (CCDC assays).

68 Because SARS-CoV-2 usually infected the lower respiratory tract, it is not easy to  
69 detect the viral nucleic acids from throat swabs with relatively lower viral load (4).  
70 Thus, qRT-PCR assays with higher sensitivity and better performance in the detection  
71 of SARS-CoV-2 is recommended in aiding the diagnosis of COVID-19 (2). However,  
72 most of the current available qRT-PCR assays were developed for emergency, a  
73 comprehensive methodological comparison among these assays remains unfulfilled.  
74 To comprehensively compare the performance of currently available qRT-PCR assays  
75 for detection of SARS-CoV-2, we evaluated the sensitivity, specificity, amplification  
76 efficiency, and linear detection ranges among IPBCAMS assays, WHO assays and  
77 CCDC assays.

## 78 **Materials and methods**

### 79 **Nucleic acid extraction**

80 Nucleic acids were extracted from a volume of 200 µl clinical samples by using  
81 NucliSens easyMag apparatus (bioMe´rieux, MarcyL'Etoile, France) according to the  
82 manufacturer's instructions. A volume of 50 µl total nucleic acid eluate for each  
83 specimen was recovered and transferred into a nuclease-free vial and either tested  
84 immediately or stored at -80°C.

### 85 **Primers and probes**

86 Sequences of primers and probes for the IPBCAMS assays were recently developed  
87 (5), while those for the WHO assays were obtained from the website of WHO  
88 ([https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef](https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2)  
89 [618c\\_2](https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2)), and those for the CCDC assays were obtained from the website of China  
90 CDC

91 ([http://www.chinacdc.cn/jkzt/crb/zl/szkb\\_11803/jszl\\_11815/202003/W020200309540](http://www.chinacdc.cn/jkzt/crb/zl/szkb_11803/jszl_11815/202003/W020200309540)  
92 843062947.pdf) (Table 1). Primers and probes were synthesized by standard  
93 phosphoramidite chemistry techniques at Qingke biotechnology Co. Ltd (Beijing,  
94 China). TaqMan probes were labeled with the molecule 6-carboxy-fluorescein (FAM)  
95 at the 5' end, and with the quencher Blackhole Quencher 1 (BHQ1) at the 3' end.  
96 Optimal concentrations of the primers and probes were determined by cross-titration  
97 of serial two-fold dilutions of each primer/probe against a constant amount of purified  
98 RNA of SARS-CoV-2.

#### 99 **TaqMan real-time RT-PCR assay**

100 The TaqMan real-time RT-PCR assays were performed by using TaqMan Fast Virus  
101 1-Step Master Mix (Thermo Fisher Scientific, MA, USA). Each 20 µl reaction mix  
102 contained 5 µl of 4×Fast Virus 1-Step Master Mix, 0.2 µl of 50 µM probe, 0.2 µl each  
103 of 50 µM forward and reverse primers, 12.4 µl of nuclease-free water, and 2 µl of  
104 nucleic acid extract. Amplifications were carried out in 96-well plates by using  
105 Bio-Rad instrument (Bio-Rad CFX96, CA, USA). Thermo-cycling conditions are as  
106 follows: 15 min at 50°C for reverse transcription, 4 min at 95°C for pre-denaturation,  
107 followed by 45 cycles of 15 sec at 95°C and 45 sec at 60°C. Fluorescence  
108 measurements were taken at 60°C of each cycle. The threshold cycle (Ct) value was  
109 determined by the point at which fluorescence exceeded a threshold limit set at the  
110 mean plus 10 standard deviations above the baseline. A result was considered positive if  
111 two or more of the SARS-CoV-2 genome targets exhibited positive results ( $Ct \leq 35$ ).  
112 A result of  $35 \leq Ct \leq 40$  was considered suspected and a repeat test was performed for  
113 result confirmation.

#### 114 **Preparation of RNA transcripts**

115 RNA transcripts for N gene and ORF 1b of SARS-CoV-2 were prepared with a  
116 plasmid pEasy-T1 (TransGen Biotech, Beijing, China) with T7 promoter before the  
117 multiple cloning sites. The plasmids inserted with viral gene regions of N and Orf1b  
118 were linearized with the restriction enzyme, BamHI, and transcribed *in-vitro* by using  
119 RiboMAX<sup>TM</sup> Large Scale RNA Production Systems (Promega, WI, USA),  
120 respectively. The concentrations of the RNA transcripts were determined by using  
121 NanoDrop (Thermo Fisher Scientific, CA, USA).

#### 122 **Results**

# **Comparison of the sensitivities, reproducibility and linear detection ranges of the three qRT-PCR assays.**

To determine the sensitivity of the three qRT-PCR assays, we measured the limit of detection (LOD) for each assay by using RNA transcript of the corresponding gene in ten-fold dilution as template (RNA transcript alone). A LOD of 10 genomic copies per reaction was observed for both the N gene assay and the ORF 1b gene assay of all the three qRT-PCR assays, although the Ct values for N gene assay of WHO assays and ORF 1b gene assay of CCDC assays were higher than 35 cycles (Table 2).

The linear detection ranges of the three qRT-PCR assays were determined by using a ten-fold dilution of the RNA transcript as template. It showed that the Ct values increased with the RNA transcript from  $10^6$  to  $10^1$  copies in the reaction in all of the three qRT-PCR assays (Table 2). Strong linear correlations were observed between Ct values and quantity of RNA transcripts with  $r^2=0.9926$ ,  $0.9750$ ,  $0.9987$  in the N gene assay, and  $r^2=0.9953$ ,  $0.9897$ ,  $0.9941$  in the ORF 1b assay of IPBCAMS assays, WHO assays, and CCDC assays, respectively. These results suggested that all of the three qRT-PCR assays exhibited linear detection ranges from  $10^6$  to  $10^1$  copies per reaction, while the WHO assays showed lower coefficient of linear correlation.

The reproducibility of the three qRT-PCR assays was assessed by measuring coefficient of variation (CV) of mean Ct values in the intra- and inter- assay. For the N gene assay, the CVs of mean Ct values from  $10^6$  to  $10^1$  copies of RNA transcript per reaction were 0.20%-1.33%, 0.46%-5.09%, 0.27%-1.97% in intra-assay, and 1.06%-2.45%, 0.96%-7.59%, 1.00%-5.51% in inter-assay of IPBCAMS assay, WHO assay, and CCDC assay, respectively. For the ORF 1b gene assay, the CVs of mean Ct values were 0.26%-4.45%, 0.29%-1.76%, 0.71%-6.52% in intra-assay, and 2.17%-5.12%, 0.30-1.57%, 2.63%-4.34% in inter-assay of IPBCAMS assays, WHO assays, and CCDC assays, respectively.

Because co-infections of respiratory viruses are common, we prepared a (v:v=1:1) mixture of the RNA transcript and a pooled total nucleic acid extract from respiratory specimens (RNA transcript + other extract) as template, to evaluate the effect of co-existed viral nucleic acids on the performance of the assays. No effect of the co-existed other viral nucleic acids on the LOD and the linear detection range was observed, although higher Ct values were generated than those of RNA transcript alone as template in all of the three qRT-PCR assays. However, the co-existed other viral nucleic acids put some effect on the efficiencies of the three qRT-PCR assays.

For the N gene assays, the efficiencies were moved from 105.82%, 107.23%, 102.21% to 110.17%, 124.32%, 119.43% in IPBCAMS assays, WHO assays, CCDC assays, respectively. For the ORF 1b assays, the efficiencies were moved from 107.71%, 121.83%, 93.80% to 109.18%, 138.43%, 100.92% in IPBCAMS assays, WHO assays, CCDC assays, respectively.

# **Comparison of the specificities of the three qRT-PCR assays**

To evaluate the potential cross-reactions with other human respiratory viruses, the three qRT-PCR assays were examined by using human respiratory samples as templates, which were positive for human coronaviruses (OC43, NL63, 229E, or HKU1), or Influenza viruses (A or B), or respiratory syncytial virus, or parainfluenza virus (1-4), or human metapneumovirus, or rhinovirus, or adenovirus, or bocavirus. No cross reaction was observed in all of the three qRT-PCR assays (data not shown), suggesting high specificity of the three qRT-PCR assays in detecting SARS-CoV-2.

# **Assay evaluation with clinical specimens**

The three qRT-PCR assays were evaluated with 25 clinical specimens (including 13 throat swabs and 12 sputum) from 25 suspected COVID-19 patients. SARS-CoV-2 was detected from 92% (23/25), 60% (15/25), 100% (25/25) by the N gene assay, and from 64% (16/25), 48% (12/25), 20% (5/25) of all enrolled clinical specimens by the ORF 1b gene assay in IPBCAMS assays, WHO assays, CCDC assays, respectively (Table 4). With respect to the sputum, SARS-CoV-2 was detected from 100% (12/12), 75% (8/12), 100% (12/12) of specimens by the N gene assay, and from 100% (12/12), 75% (8/12), 41.7% (5/12) of specimens by the ORF 1b gene assay in IPBCAMS assays, WHO assays, CCDC assays, respectively. About the throat swabs, SARS-CoV-2 was detected from 84.6% (11/13), 53.8% (7/13), 100% (12/12) of specimens by the N gene assay, and from 30.8% (4/13), 30.8% (4/13), 0% (0/13) of specimens by the ORF 1b gene assay in IPBCAMS assays, WHO assays, CCDC assays, respectively. These results demonstrated that the N gene assay performed better than the corresponding ORF 1b gene assay of all the three qRT-PCR assays, the N gene assay in CCDC assays and ORF 1b gene assay in IPBCAMS assays performed better than the other assays.

# **Discussion**

Rapid and accurate detection of SARS-CoV-2 represent a fast-growing global demand, which could be met by TaqMan real time RT-PCR (qRT-PCR). However, the current available TaqMan qRT-PCR assays for SARS-CoV-2 are varied in performance,

191 including sensitivity, specificity, reproducibility, linear detection ranges, etc. Due to  
 192 that relative lower viral load in upper respiratory tract, reliable qRT-PCR assays for  
 193 the detection of SARS-CoV-2 are required. We thus compared the performance of  
 194 three currently wide-applied qRT-PCR assays in the detection of SARS-CoV-2.

195 Sensitivity is the primary demand in the detection of respiratory viruses (6). All of the  
 196 three qRT-PCR assays could provide a LOD of 10 genomic copies per reaction with a  
 197 detection range from  $10^6$ - $10^1$  genomic copies per reaction. The Ct value at 10  
 198 genomic copies per reaction in the ORF 1b gene assay of CCDC assays was higher  
 199 than 35. These results suggested that most of the three qRT-PCR assays provide high  
 200 sensitivity and wide linear detection range in detecting SARS-CoV-2, except a  
 201 relative lower sensitivity observed in the ORF 1b gene assay of CCDC assays.

202 Specificity is also essential in the detection of SARS-CoV-2, because of common  
 203 co-infections with other respiratory viruses and high host DNA background in throat  
 204 swabs (7-9). We evaluated the specificity of the three qRT-PCR assays with  
 205 respiratory specimens positive for other common respiratory viruses. No cross  
 206 reaction was observed, demonstrating high specificity of the three qRT-PCR assays in  
 207 detection of SARS-CoV-2.

208 We next evaluated the reproducibility of the three qRT-PCR assays by measuring  
 209 coefficient of variation (CV) of mean Ct values in intra- and inter- assay (10). The N  
 210 gene assay in IPBCAMS assays and ORF 1b gene assay in WHO assays exhibited a  
 211 relative better reproducibility with lower intra- and inter- assay CVs, which were not  
 212 affected by the co-existed nucleic acids of other respiratory viruses.

213 Efficiency is another key parameter of qRT-PCR, reflecting the binding efficiency of  
 214 primers & probe to template and the amplification efficiency of the PCR system(11).  
 215 Most of the qRT-PCR assays provided good efficiency, except an abnormal efficiency  
 216 of 121.83% observed in the ORF 1b gene assay of WHO assays. An exceptionally  
 217 high efficiency indicates an increased risk of false positive (12). The co-existed  
 218 nucleic acids of other respiratory viruses increased the efficiency of all the three  
 219 qRT-PCR assays, suggesting potential increased risk of cross-reactions between the  
 220 primers & probe and background nucleic acids.

221 We finally evaluate the performance of the three qRT-PCR assays with clinical  
 222 specimens from suspected SARS-CoV-2 infected patients (13). Possibly because of  
 223 the lower viral load in upper respiratory tract (4), the detection rate of SARS-CoV-2  
 224 was lower in throat swabs than in sputum by all of the three assays. Meanwhile, the N



gene assay performed better than the corresponding ORF 1b gene assay in all of the three qRT-PCR assays. For the N gene assay, IPBCAMS assays and CCDC assays performed better than WHO assays, both of which could detect SARS-CoV-2 from more than 90% of the suspected specimens. For the ORF1b gene assay, IPBCAMS assays performed better than WHO assays and CCDC assays, with a detection rate of 64%.

In conclusion, we performed methodological evaluations on three widely-applied qRT-PCR assays for the detection of SARS-CoV-2. Although most of the evaluated assays exhibited good sensitivity, specificity, reproducibility and wide linear detection range, performance test with clinical specimens from suspected COVID-19 patients suggested that the N gene assay in IPBCAMS assays and CCDC assays, and the ORF 1b gene assays in IPBCAMS assays were the preferred qRT-PCR assays for accurate detection of SARS-CoV-2.

# **Data availability**

The original data will be available upon request.

# **Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

# **Acknowledgements**

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**Table 1. Primers and probes of the three qRT-PCR assays**

Assay	Primer/probe	Sequence (5'-3')	Genomic location*	Amplicon
N gene assay	IPBCAMS assays	Forward	AACACAAGCTTTCGGCAGAC	195 bp
		Reverse	ACCTGTGTAGGTCAACCACG	
		Probe	CAGCGCTTCAGCGTTCTTCGGAATGTCGC	
	WHO assays	Forward	CACATTGGCACCCGCAATC	127 bp
		Reverse	GAGGAACGAGAAGAGGCTTG	
		Probe	ACTTCCTCAAGGAACAACATTGCCA	
	CCDC assays	Forward	GGGGAAGTTCTCTGCTAGAAT	98 bp
		Reverse	CAGACATTTTGCTCTCAAGCTG	
		Probe	TTGCTGCTGCTTGACAGATT	
	IPBCAMS assays	Forward	ACGGTGACATGGTACCACAT	215 bp
		Reverse	CTAAGTTGGCGTATACGCGT	
		Probe	TACACAATGGCAGACCTCGTCTATGC	
ORF 1b gene assay	WHO assays	Forward	GTGARATGGTCATGTGTGGCGG	99 bp
		Reverse	CARATGTTAAASACACTATTAGCATA	
		Probe	CAGGTGGAACCTCATCAGGAGATGC	
	CCDC assays	Forward	CCCTGTGGGTTTTACACTTAA	118 bp
		Reverse	ACGATTGTGCATCAGCTGA	
		Probe	CCGTCTGCGGTATGTGGAAAGGTTATGG	

Numbering according to a reference genome of SARS-CoV-2 (MN908947.3)

**Table 2. Reproducibility (Coefficient of Variation, %) of the three qRT-PCR assays**

Assay			Copy number of RNA transcript					
			$1 \times 10^6$	$1 \times 10^5$	$1 \times 10^4$	$1 \times 10^3$	$1 \times 10^2$	$1 \times 10^1$
N gene assay	IPBCAMS assays	Intra-assay	0.52*	1.33	0.37	0.46	0.20	1.25
		Inter-assay	1.06	2.45	1.49	1.32	1.37	1.45
	WHO assays	Intra-assay	1.08	1.19	1.12	0.87	0.46	5.09
		Inter-assay	7.59	2.94	2.78	6.60	0.96	3.77
	CCDC assays	Intra-assay	0.52	0.54	0.27	0.74	0.41	1.97
		Inter-assay	1.56	1.20	5.51	1.00	1.40	2.89
ORF 1b gene assay	IPBCAMS assays	Intra-assay	0.73	0.26	1.10	1.30	4.45	3.36
		Inter-assay	4.66	3.85	2.77	2.17	5.12	3.50
	WHO assays	Intra-assay	0.57	0.47	0.88	0.41	0.29	1.76
		Inter-assay	1.57	0.30	0.87	0.69	0.55	1.23
	CCDC assays	Intra-assay	1.66	0.78	0.71	0.92	2.45	6.52
		Inter-assay	0.52	0.54	0.27	0.74	0.41	1.97

The coefficient of variation was calculated by standard deviation of the Ct values of a RNA dilution divided by the mean Ct values of the same RNA dilution.

**Table 3. Efficiency of the three qRT-PCR assays**

Assay	Template	Mean Ct values at quantified copy number of RNA transcript							Slope <sup>a</sup>	Efficiency (%) <sup>b</sup>
		1×10 <sup>6</sup>	1×10 <sup>5</sup>	1×10 <sup>4</sup>	1×10 <sup>3</sup>	1×10 <sup>2</sup>	1×10 <sup>1</sup>			
N gene assay	IPBCAMS	RNA transcript <sup>d</sup> alone	17.63±0.09 <sup>c</sup>	21.99±0.29	24.08±0.09	28.25±0.13	31.00±0.06	33.73±0.25	-3.19	105.82
	assays	RNA transcript + other viruses	19.40±0.19	22.40±0.04	26.38±0.09	29.98±0.07	32.17±0.28	34.51±0.26	-3.10	110.17
	WHO assays	RNA transcript alone	18.44±0.19	22.65±0.27	26.78±0.32	29.60±0.26	32.68±0.15	33.97±1.73	-3.16	107.23
		RNA transcript + other viruses	19.51±0.15	24.83±0.36	26.59±0.29	29.62±0.54	32.62±0.70	34.19±0.51	-2.85	124.32
	CCDC assays	RNA transcript alone	17.17±0.09	20.71±0.11	23.94±0.07	27.57±0.20	30.37±0.12	33.53±0.50	-3.27	102.21
		RNA transcript + other viruses	18.93±0.16	23.79±0.20	25.66±0.23	29.58±0.52	31.92±0.16	33.81±0.87	-2.93	119.43
ORF 1b gene assay	IPBCAMS	RNA transcript alone	18.64±0.14	22.20±0.06	25.73±0.28	28.83±0.37	31.90±1.42	34.22±1.15	-3.15	107.71
	assays	RNA transcript + other viruses	19.45±0.06	22.98±0.13	25.88±0.17	29.37±0.12	32.83±0.40	34.65±2.12	-3.12	109.18
	WHO assays	RNA transcript alone	18.51±0.11	21.60±0.10	25.05±0.22	28.27±0.12	30.78±0.09	32.57±0.57	-2.89	121.83
		RNA transcript + other viruses	19.46±0.09	22.58±0.13	25.75±0.19	28.20±0.20	30.03±0.70	33.04±0.14	-2.65	138.43
	CCDC assays	RNA transcript alone	18.80±0.31	21.96±0.17	24.76±0.18	28.06±0.26	32.47±0.79	36.16±2.36	-3.48	93.80
		RNA transcript + other viruses	18.67±0.04	21.54±0.11	24.79±0.03	28.28±0.04	31.09±0.98	35.33±0.59	-3.30	100.92

<sup>a</sup> Slope was generated by fitting of the scatter with Excel 2010.

<sup>b</sup> Efficiency =  $10^{(-1/\text{slope})} - 1$ .

<sup>c</sup> Values shown are the mean of triplicate samples ± standard deviation.

<sup>d</sup> “RNA transcript” represents the *in vitro* transcribed RNA of the corresponding genes of SARS-CoV-2. “other viruses” represents the pooled RNA extracted from 15 human respiratory specimens by using Trizol. “RNA transcript + other viruses” represents a 1:1 (v/v) mixture of these two components.

**Table 4. Evaluation of the three qRT-PCR assays with clinical specimens**

Specimen ID	Specimen type	N gene assay			ORF 1b gene assay		
		IPBCAMS	WHO	CCDC	IPBCAMS	WHO	CCDC
TS98	Throat swab	35.79	NA	35.42	NA	NA	NA
TS101	Throat swab	33.48	NA	34.24	NA	NA	NA
TS103	Throat swab	NA	NA	34.68	NA	NA	NA
TS105	Throat swab	31.5	35.76	31.64	NA	NA	NA
TS108	Throat swab	33.35	NA	32.11	33.36	NA	NA
TS110	Throat swab	29.99	31.73	29.1	33.57	NA	NA
TS165	Throat swab	27.34	30.46	28.14	31.06	27.84	NA
TS168	Throat swab	NA	NA	34.97	NA	NA	NA
TS169	Throat swab	33.34	NA	34.04	NA	34.2	NA
TS187	Throat swab	34.5	39.2	33.03	NA	NA	NA
TS188	Throat swab	35.03	35.9	33.57	NA	24.07	NA
TS189	Throat swab	31.16	35.43	31.21	34.04	30.92	NA
TS190	Throat swab	32.84	34.02	32.56	NA	NA	NA
TY1	Sputum	27.35	29.44	27.6	30.98	27.33	NA
TY2	Sputum	29.38	31.26	29.06	32.32	28.72	NA
TY3	Sputum	31.85	NA	31.3	35.84	NA	NA
TY4	Sputum	22.99	25.57	22.08	27.42	24.12	35.99
TY6	Sputum	25.51	27.52	25.58	29.03	25.58	41.54
TY7	Sputum	26.9	30.21	27.4	30.05	27.3	45.26
TY8	Sputum	29.21	31.87	30.06	33.65	29.84	NA
TY9	Sputum	26.29	28.45	26.34	30.69	26.03	46.34
XT1	Sputum	25.74	27.26	25.3	29.82	26.34	45.9
XT2	Sputum	31.57	NA	30.95	34.19	NA	NA
XT3	Sputum	31.14	NA	32.02	35.02	NA	NA
XT4	Sputum	32.67	NA	31.71	34.26	NA	NA
account (%) of positive		23 (92%)	15 (60%)	25 (100%)	16 (64%)	12 (48%)	5(20%)

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