Evaluating the SARS-CoV-2 Specific Antibodies in Saliva

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Abstract

Molecular assays on nasopharyngeal swabs act as the confirmatory test in CoronaVIrus Disease (COVID-19) diagnosis. Despite massive efforts had been made, the high technicalities of nasopharyngeal sampling and molecular assays limit the testing capabilities. Currently, the use of saliva for diagnosis has been recently suggested for COVID-19 diagnostic testing. In a recent research, salivary IgA was associated with the presence of pneumonia, which might illuminate that salivary IgA was independent from serum immunoglobulins. In this study, a total of 44 patients diagnosed with COVID-19 in the Third People's Hospital of Shenzhen were enrolled. Saliva specimens and serum specimens were obtained at different time points and the immunoglobulins against the SARS-CoV-2 was measured. The results showed that saliva IgA presented higher COI value than IgG and IgM. In matched saliva and serum samples, all saliva presented lower IgG level than serum, and only one saliva sample presented higher IgM level. The conversion rates of saliva IgA and the detection of viral nucleic acid were analyzed in the first and second week after hospitalization. The positive rates were obviously increased when combining the saliva IgA and viral nucleic acid detection. Saliva IgA could serve as useful index for early diagnosis of COVID-19.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of Interest Disclosure

The authors declare no conflict of interest.

Ethics Approval Statement

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Abstract

Molecular assays on nasopharyngeal swabs act as the confirmatory test in CoronaVIrus Disease (COVID-19) diagnosis. Despite massive efforts had been made, the high technicalities of nasopharyngeal sampling and molecular assays limit the testing capabilities. Currently, the use of saliva for diagnosis has been recently suggested for COVID-19 diagnostic testing. In a recent research, salivary IgA was associated with the presence of pneumonia, which might illuminate that salivary IgA was independent from serum immunoglobulins. In this study, a total of 44 patients diagnosed with COVID-19 in The Third People's Hospital of Shenzhen were enrolled. Saliva specimens and serum specimens were obtained at different time points and the immunoglobulins against the SARS-CoV-2 was measured. The results showed that saliva IgA presented higher

COI value than IgG and IgM. In matched saliva and serum samples, all saliva presented lower IgG level than serum, and only one saliva sample presented higher IgM level. The conversion rates of saliva IgA and the detection of viral nucleic acid were analyzed in the first and second week after hospitalization. The positive rates were obviously increased when combining the saliva IgA and viral nucleic acid detection. Saliva IgA could serve as useful index for early diagnosis of COVID-19.

Keywords

COVID-19, SARS-CoV-2, immunoglobulins, saliva IgA, diagnose

Introduction

Outbreak pneumonia announced in Wuhan, China, in December 2019, had its causative factor classified as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The current CoronaVIrus Disease (COVID-19) pandemic is developing rapidly into a dramatically devastating public health crises in recent history. By April 2021, reported cases exceeded 147 million worldwide, with at least 3,144,381 deaths and 110.27 million people recovered. Molecular assays on nasopharyngeal swabs act as the confirmatory test in COVID-19 diagnosis. Despite massive efforts had been made, the high technicalities of nasopharyngeal sampling and molecular assays limit the testing capabilities [1]. The positive rate of RT-PCR RNA detection was 63% in nasal swabs and only 32% in pharyngeal swabs [2]. Serological assays play an important supporting role in COVID-19 clinical diagnosis. Generally, IgM and IgG-based assays are the gold standard for serological diagnosis in COVID-19 [3]. Nowadays, SARS-CoV-2 S1 and N antigens have been detected in the serum of SARS-CoV-infected patients [4], which might identify active infection and monitor disease progression in COVID-19 patients.

Currently, nasopharyngeal swabs are main recommended upper respiratory tract specimen types for COVID-19 diagnostic testing, while the use of saliva for the diagnosis of the disease has been recently suggested [5,6]. Saliva specimens could be conveniently obtained by telling patients to spit into a sterile container, which is non-painful and non-stressful for patients [7]. Also, the collection of saliva is non-invasive and greatly minimizes the exposure of healthcare workers to COVID-19. Some researchers have concluded that detection of SARS-CoV-2 salivary antibodies could serve as a non-invasive alternative to serological testing for monitoring of SARS-CoV-2 infection and seropositivity at population scale [8]. Saliva is secreted by salivary glands which is characteristic in abundant IgA. Usually, salivary IgG and IgM concentrations are much lower than in serum [9]. It has been hypothesized that both salivary IgG and IgM are derived from blood, whereas IgA is mainly produced by the salivary glands [10].

In a recent COVID-19 research, salivary IgA was associated with the presence of pneumonia, but not associated with serum immunoglobulins [11]. This might illuminate that salivary IgA was independent from serum immunoglobulins. In this study, we measured saliva specimens and serum specimens from 44 COVID-19 patients and 24 negative-control patients. The associations between saliva and serum immunoglobulins were described and the potential of saliva IgA in COVID-19 diagnosis was assessed.

Results

Patients diagnosed with COVID-19 from August 1st to September 1st, at The Third People's Hospital of Shenzhen were enrolled in this study (n = 44). The characteristics including age, gender and disease severity were listed in **Table 1** . Most patients were male and asymptomatic. The average age of the patients was 43 years (range, 22-62 years). Saliva and serum from patients were collected and the levels of IgA, IgG and IgM were measured. The highest COI values of each patient was used to present the immunoglobulin level in saliva or serum. As shown in**Fig. 1** and **Table 2** , 14 patients presented positive IgA in saliva, while 7 and 4 patients presented positive IgG and IgM, respectively. Moreover, IgA presented higher COI value than IgG and IgM in saliva ($p = 0.0128$ and $p = 0.0297$, respectively). 24 negative-control patients were selected randomly from inpatient departments as negative control. IgA, IgG or IgM in saliva and serum specimens were all negative (**Fig. 2**).

Saliva and serum which were collected on the same day or two consecutive days were analyzed as matched

samples (n=15) **(Table 3)** . As shown in **Fig. 3A-C** , 5 saliva specimens presented higher IgA level than matched serum. In general, IgA in saliva specimens showed a roughly same level with serum (saliva, 11 positive vs 4 negative; serum, 10 positive vs 5 negative). IgG and IgM levels in saliva specimens were lower than those in serum ($p < 0.0001$ and $p = 0.0444$, respectively). All saliva presented lower IgG level than serum (saliva, 5 positive vs 10 negative; serum, 15 positive vs 0 negative), and only one saliva specimen presented higher IgM level (saliva, 3 positive vs 12 negative; serum, 5 positive vs 10 negative). No clear correlation was observed among IgA, IgG and IgM positive samples (**Fig. 3D**).

To investigate whether the test of saliva IgA could improve the diagnostic power of COVID-19 patients, the conversion rates of saliva IgA and the detection of viral nucleic acid were analyzed in the first and second week after hospitalization (n=39) (**Table 4**). Though all the patients were hospitalized with positive nucleic acid result at the beginning, the positive rate was as low as 35.90% in the first week, and then 12.82% in the second week. The positive rates were obviously increased with saliva IgA.

Discussion

This study provides data about the use of saliva for the detection of SARS-CoV-2 specific antibodies of samples from COVID-19 patients. The present study was conducted at The Third People's Hospital of Shenzhen in September 2020, so most patients enrolled were in the recovery phase of the disease. This may explain why the percentage of positive rate of SARS-CoV-2 nucleic acid in our inpatients series was low.

Saliva has been used over decades for evaluating human health. It offers several advantages as a diagnostic medium in that it is a noninvasive, painless, safe, and convenient specimen. Whereas some consider phlebotomy specimens to be too invasive and uncomfortable, saliva sampling is widely accepted, particularly among vulnerable or difficult-to-reach populations, and could facilitate home-based self-collection [12,13]. Pisanic N et al. have tested SARS-CoV-2 specific IgA, IgG and IgM in saliva specimens with considerable detection rate [8]. In an Australian family case, saliva antibodies were also detected from all family members [14]. In our study, despite the low detection rate, IgA, IgG and IgM were all detectable in saliva specimens.

Secretory IgA is a principal component of mucosal immunity, and can easily be measured in saliva[15]. In a recent research, IgA has been proved to be the dominant antibody in early SARS-CoV-2-specific humoral responses [16]. Salivary IgA antibody response was reported to be particularly prevalent in younger individuals with mild SARS-CoV-2 infection [17]. Similarly, in our results, the level and detection rate of IgA in saliva were obviously higher than IgG and IgM. The correlation between saliva and serum SARS-CoV-2-specific antibodies has been assessed that IgA, IgG and IgM levels in matched saliva and serum samples were all significantly correlated [8]. While, IgA levels in the saliva was reported to exhibit the poorest correlation with IgA levels in the serum[18]. In our results, levels of IgG and IgM in saliva were obviously lower than in serum. When comparing the IgA level in paired saliva and serum samples, no clear correlation could be drawn.

Recently, saliva has been proposed as a suitable specimen for the diagnosis of COVID-19, and the collection method would reduce the exposure risk of frontline health workers which is one of major concerns in primary healthcare settings [19]. SARS-CoV-2 RNA could remain detectable in saliva over a 1-week period but the test is unstable and vulnerable [20,21]. Neutralizing IgA was reported to remain detectable in saliva for a longer time (days 49 to 73 post symptoms) than in serum [16]. Our results have shown that testing for antibodies against SARS-CoV-2 was sensitive in saliva samples, providing an easy, noninvasive option for detection of prior infection. The combination of antibody test on saliva and traditional molecular assays on nasopharyngeal swabs could approve the diagnosis ability. Also, the increased salivary IgA has been proposed to serve as a biomarker to identify patients at an elevated risk of clinical deterioration in COVID-19 [15]. All these evidences suggested that the IgA in saliva could play an important role in COVID-19 diagnosis.

This study has several limitations. First, it is well known that the antibody concentration in human saliva is orders of magnitude lower than in blood or serum. Assays with exquisite analytical sensitivity and capable of detecting high signal over background noise were demanded [22]. Additionally, our sample set was not large enough, especially lacking the samples at early time points, weakening the robustness of our findings in

saliva. Future studies could improve on the robustness by including a larger sample size at all time points.

Materials and Methods

Patients

A total of 44 patients diagnosed with COVID-19 based on the World Health Organization's interim guidance, from August 1st to September 1st, 2020, at The Third People's Hospital of Shenzhen were enrolled in this study. 24 negative-control patients with no SARS-CoV-2 infection were selected randomly from inpatient departments at the same time. The study was approved by the Ethics Committee of The Third People's Hospital of Shenzhen. Written informed consents were obtained from all participants enrolled in the study.

Immunoglobulin measurement

A total 180 saliva specimens and 181 peripheral blood specimens were obtained from COVID-19 patients with RT-PCR-confirmed prior SARS-CoV-2 infection, at different time points during hospitalization. Saliva specimens and peripheral blood specimens were also obtained from negative-control patients. The serum specimens were obtained from the supernatant of centrifuged peripheral blood at 3500 rpm for 5 min. The saliva specimens were also centrifuged and the supernatants were obtained to perform immunoglobulin detection. Before test, all specimens were inactivated at 56 for 30 min. Immunoglobulins against the SARS-CoV-2 surface spike protein receptor-binding domain (RBD) was measured by chemiluminescence kit (IgA, IgG, and IgM, Beijing Wantai Biotech, China) according to the manufacturer's instruction in the Caris200 automatic chemiluminescence instrument. Fluorescence intensity was used to measure antibody concentration. The relative fluorescence of sample to control (COI) was used to estimate the result. The results [?] 1 COI are positive, and the results < 1 COI are negative. The peak COI values of immunoglobulins were analyzed based on the results of relative fluorescence measurement.

Real-time PCR detection of SARS-CoV-2

Over 240 swab samples were obtained from the upper respiratory tracts of participants at different time points throughout hospitalization. The presence of SARS-CoV-2 was detected by qRT-PCR assay as previously reported. Briefly, the nucleocapsid protein and open reading frame 1ab were amplified and examined with two pairs of primers. Each sample was detected in triplicate with positive and negative controls. The diagnostic criteria were based on the recommendations by the National Centers for Disease Control and Prevention of China.

Statistical analysis

The statistical analysis was performed in SPSS software version 22.0. All statistical figures were drawn in GraphPad Prism software version 8.0.1. Student's t-test was used for comparing the difference between different antibodies in saliva. Paired t-test was used to analyze the difference of antibody COI between serum and saliva.

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Institutional Review Board Statement

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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Tables

Table 1. Characteristics of enrolled patients (n = 44).

Patient	Gender	Age	Disease severity	Patient	Gender	Age	Disease severity
p1	male	50	asymptomatic	p23	male	51	asymptomatic
p2	male	33	asymptomatic	p24	male	35	asymptomatic
p3	male	48	asymptomatic	p25	male	43	asymptomatic
p4	male	43	asymptomatic	p26	male	40	asymptomatic
p5	male	33	asymptomatic	p27	male	48	asymptomatic
p6	male	48	asymptomatic	p28	male	51	asymptomatic
p7	male	32	asymptomatic	p29	male	40	asymptomatic
p8	female	37	severe	p30	male	41	asymptomatic
p9	male	44	asymptomatic	p31	male	46	asymptomatic
p10	male	59	asymptomatic	p32	male	30	asymptomatic

Patient	Gender	Age	Disease severity	Patient	Gender	Age	Disease severity
p11	male	31	asymptomatic	p33	male	43	asymptomatic
p12	male	33	asymptomatic	p34	female	62	moderate
p13	male	36	asymptomatic	p35	male	34	asymptomatic
p14	male	44	asymptomatic	p36	male	32	asymptomatic
p15	male	28	asymptomatic	p37	male	42	asymptomatic
p16	male	29	asymptomatic	p38	male	50	asymptomatic
p17	male	51	asymptomatic	p39	male	54	asymptomatic
p18	female	52	asymptomatic	p40	male	52	asymptomatic
p19	male	43	asymptomatic	p41	male	46	asymptomatic
p20	female	53	asymptomatic	p42	male	22	asymptomatic
p21	male	34	asymptomatic	p43	male	39	moderate
p22	male	40	asymptomatic	p44	male	29	asymptomatic

Table 2. Positive rate of immunoglobulins in saliva.

Immunoglobulin	Positive (+)	Negative (-)	total	Positive rate (%)
IgA	14	30	44	31.82
IgG	7	37	44	15.91
IgM	4	40	44	9.09

Table 3. The collection time and results of paired serum and saliva specimens.

serum	serum	serum	serum	serum	serum	serum	saliva	saliva	sa
collection time (days)	IgA	IgA	IgG	IgG	IgM	IgM	collection time (days)	IgA	Ig
2	0.98	(-)	8.08	(+)	4.52	(+)	3	0.35	(-
2	2.14	(+)	12.6	(+)	5.17	(+)	3	1.03	(-
4	3.93	(+)	7.55	(+)	0.2	(-)	3	1.32	(-
5	1.88	(+)	20.68	(+)	6.82	(+)	4	1.75	(-
5	0.37	(-)	8.53	(+)	0.85	(-)	4	1.12	(-
7	1.15	(+)	2.24	(+)	4.8	(+)	6	1.13	(-
8	4.85	(+)	18.82	(+)	3.43	(+)	7	0.14	(-
8	4.5	(+)	5.64	(+)	0.16	(-)	8	4.7	(-
9	1.21	(+)	17.74	(+)	0.24	(-)	10	1.47	(-
11	1.36	(+)	10.3	(+)	0.08	(-)	10	5.21	(-
11	0.66	(-)	3.49	(+)	0.45	(-)	12	0.09	(-
12	2.42	(+)	12.36	(+)	0.28	(-)	12	0.41	(-
13	0.71	(-)	12.98	(+)	0.41	(-)	12	1.23	(-
14	6.06	(+)	5.66	(+)	0.26	(-)	13	4.15	(-
19	0.99	(-)	3.8	(+)	0.12	(-)	20	1.03	(-

Note:

The first column of each type of immunoglobulins was COI value and the second column was the qualitative result. (+) means positive and (-) means negative.

Table 4. Positive detection rate of SARS-CoV-2 nucleic acid and saliva IgA at different time periods.

Time (days)	RNA	RNA	saliva IgA	saliva IgA	RNA or saliva IgA	RNA or saliva IgA
	n	positive rate (%)	n	positive rate (%)	n	positive rate (%)
1-7	14	35.90	6	15.38	19	48.72
8-14	5	12.82	3	7.69	8	20.51
1-14	15	38.46	8	20.51	20	51.28

Figures

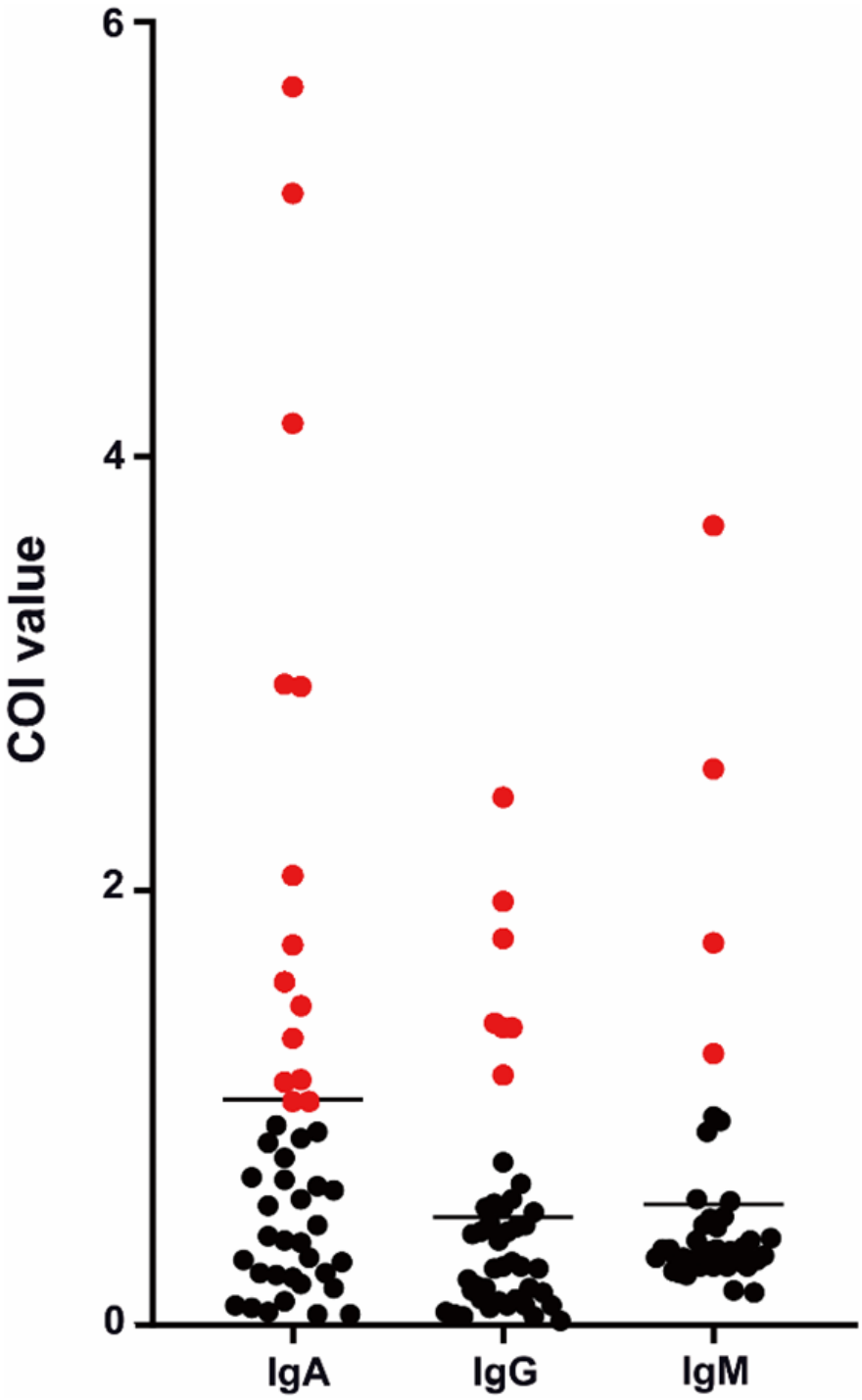


Figure 1 Peak levels of saliva immunoglobulins in COVID-19 patients.

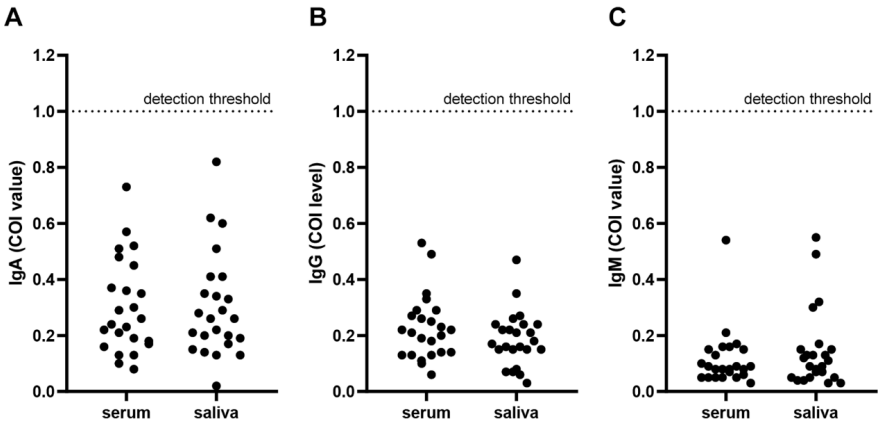
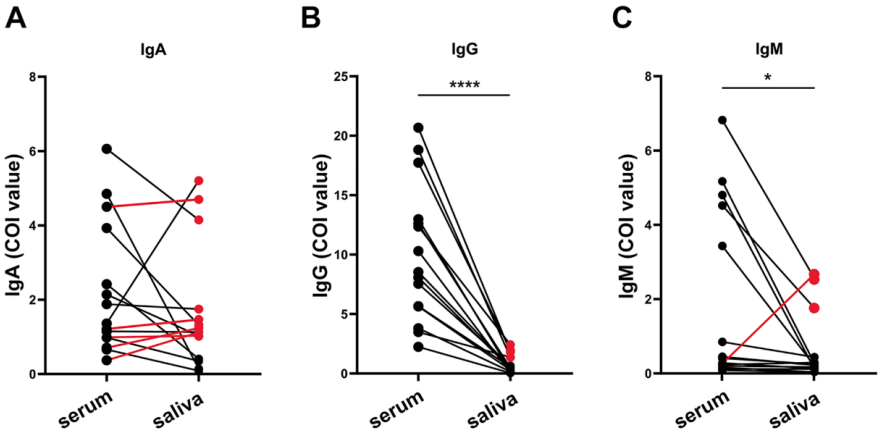


Figure 2 Immunoglobulins in serum and saliva specimens from negative-control patients.



D

Saliva Specimen	IgA	IgG	IgM
1	+	-	+
2	+	+	+
3	+	-	-
4	+	-	-
5	-	-	+
6	+	-	-
7	-	+	-
8	+	-	-
9	+	-	-
10	+	-	-
11	+	-	-
12	-	+	-
13	+	+	-
14	-	+	-
15	+	-	-

Figure 3 Comparison of immunoglobulins in serum and saliva.

Figure Legends

Figure 1 Peak levels of saliva immunoglobulins in COVID-19 patients.

Each point presented the highest measured COI value of immunoglobulin in saliva of each patients. Positive results were colored in red.

Figure 2 Immunoglobulins in serum and saliva specimens from negative-control patients.

Each point presented the COI value of IgA (A), IgG (B) and IgM (C) in serum or saliva specimens of each negative-control patient. The detection threshold was marked in each figure at COI = 1.

Figure 3 Comparison of immunoglobulins in serum and saliva.

A-C: Each point presented a measured COI value of immunoglobulin in serum or saliva. Two points with line were paired samples collected from same patient. Red points presented positive result. Red lines meant the level of immunoglobulin in saliva was higher than the corresponding serum. D: The list of antibody levels 15 saliva samples.

+ means positive and – means negative.