**Virologica Sinica**

**Supplementary Data**

**Saliva-based point-of-care testing techniques for COVID-19 detection**

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**Patient recruitment**

A cohort of 100 COVID-19 patients (62 males, 38 females) was enrolled at the Wuhan Jinyintan hospital in Hubei province before July 2020, with an age range of 13–82 years (average, 52 ± 14 years). The patients were asked to simultaneously provide serum and saliva specimens. Subsequently, SARS-CoV-2-specific IgG antibody levels were detected and quantified in both samples by an enzyme-linked immunosorbent assay (ELISA) kit (provided by Wuhan KeyuanAnbo Biotechnology Co. Ltd., Wuhan, China).

**Sample collection**

Saliva samples were collected by chewing on a cotton swab with a saliva collection device (Salivette, Germany) for 3–5 min, or until saturation. The saturated cotton swab was then inserted into the storage tube, capped, and stored at −20 °C until processing. Blood samples were collected into serum separator tubes (SST). Both serum and saliva were heated at 50 °C and inactivated for 30 min and then stored at 4 °C until analysis. Saliva was separated from the cotton swab through centrifugation (10 min, 3010 ×*g*) and transferred to the attached 2-mL cryovial.

**Laboratory methods**

The saliva and serum samples were centrifuged for 20 min at 3010 ×*g* and the supernatants were extracted. SARS-CoV-2 IgG antibodies were measured in both samples using an ELISA kit (Wuhan KeyuanAnbo Biotechnology Co. Ltd., Wuhan, China). To accurately measure the level of IgG antibodies, serum samples were diluted 1:400 with the provided sample buffer, while the saliva samples were used without further processing. Serum and saliva samples were added to wells, incubated at 37 °C for 1 h, then washed with buffer containing 2% serum albumin. Thereafter, mouse anti-human antibody supplemented with horseradish peroxidase was added and incubated for 30 min at 37 °C. Next, solution A (containing carbamide peroxide) and solution B (containing tetramethylbenzidine, TMB) were added and mixed. The colorimetric reaction proceeded for 15 min at room temperature and 50 µL of H2SO4 was added into the wells. Finally, the optical density (OD) was recorded by an automated reader (Multimode Plate Reader, PerkinElmer).

**Relationship between optical density and IgG titers**

As shown in Supplementary Fig. S1, the serum samples were diluted with a known IgG titer (TCID50, 1280) (diluted from 1:100 to 1:12,800) to determine the corresponding relationship between OD and titers. The titers were linearly proportional to the OD within the range of 0.1–1.6 (dilution ratio ranges from 1:800 to 1:12,800, Y = 0.109 + 1.751X, R2 = 0.9891), indicating that the OD has a good corresponding relationship with the titers.

**Data stability**

The testing stability of the ELISA kits was evaluated based on the repeated detection of IgG titers. Supplementary Table S1 shows that the relative standard deviations (RSD) were less than 10% for repeated IgG detections at all tested concentrations, indicating that this testing had good reproducibility.

**Equation used in the present study**

The cutoff value was determined as the mean optical density (OD) value for the negative control plus 0.093. The true-positive, false-positive, false-negative, and true-negative rates were estimated and used to calculate the diagnostic values (sensitivity, specificity, and others). To calculate the specificity and sensitivity of saliva-based antibody detection, the serum antibody level was regarded as the “gold standard.” The test results for 100 COVID-19 convalescent patients are shown in Supplementary Table S2.

Equation S1. The cutoff value was determined as the mean optical density value for the negative control plus 0.093 (given by the ELISA kit manufacturer).

Equation S2. Calculation of the sensitivity of saliva-based antibody detection.

Equation S3. Calculation of the specificity of saliva-based antibody detection.

**Supplementary Table S1** Different dilution ratios were analyzed five times with the ELISA kit, showing RSD values of ＜ 10%.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dilution ratio | OD | | | | | Mean | STD | RSD (%) |
| 800 | 1.655 | 1.639 | 1.626 | 1.651 | 1.673 | 1.649 | 0.0176 | 1.06 |
| 1600 | 0.846 | 0.820 | 0.893 | 0.874 | 1.035 | 0.894 | 0.0838 | 9.37 |
| 3200 | 0.427 | 0.423 | 0.418 | 0.470 | 0.497 | 0.434 | 0.0358 | 8.23 |

*OD*, optical density; *STD*, standard deviation; *RSD*, relative standard deviation.

**Supplementary Table S2** Test results of 100 COVID-19 convalescent patients

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | COVID-19 patients, n | | Total, n |
| Positive | True positive | False positive | 83 |
| 82 | 1 |
| Negative | False negative | True negative | 17 |
| 12 | 5 |
| Total | 94 | 6 | 100 |

图片包含 黑暗, 游戏机, 男人, 房间

描述已自动生成

**Supplementary Fig. S1** Relationship between optical density and IgG titers.