Evaluation of a Commercial Culture-free Neutralization Antibody Detection Kit for Severe Acute Respiratory Syndrome-Related Coronavirus-2 and Comparison with an Anti-RBD ELISA Assay

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ABSTRACT

Background: SARS-CoV-2 surrogate neutralization assays that bypass the need for viral culture offer substantial advantages regarding throughput and cost. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit (Genscript) is the first such commercially available assay, detecting antibodies that block RBD/ACE-2 interaction. We aimed to evaluate cPass to inform its use and assess its added value compared to anti-RBD ELISA assays.

Methods: Serum reference panels were used to compare cPass to plaque-reduction neutralization test (PRNT) and a pseudotyped lentiviral neutralization assay for detection of neutralizing antibodies. We assessed the correlation of cPass with an ELISA detecting anti-RBD IgG, IgM, and IgA antibodies at a single timepoint and across intervals from onset of symptoms of SARS-CoV-2 infection.

Results: Compared to PRNT-50%, cPass had 100% sensitivity (95%CI 82-100) and 95% specificity (76-100). Sensitivity was also very high compared to the pseudotyped lentiviral neutralization assay, but specificity was lower, ranging from 17-70%. Highest agreement between cPass and ELISA was for anti-RBD IgG ($r=0.851$ at 0-6 weeks; $r=0.798$ at > 6 weeks). Anti-RBD IgG diagnostic accuracy for detection of neutralizing antibodies was essentially identical to that of cPass.

Conclusions: The added value of cPass compared to an IgG anti-RBD ELISA was not supported by these results.

Key Words: “SARS-CoV-2”, “COVID-19”, “Diagnosis”, “Serology”, “ELISA”, “Neutralization Test”, “Neutralizing Antibodies”, “Immunity”
INTRODUCTION

Use cases for serological testing for prior exposure to Severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) have been reviewed in detail (1, 2). Despite a rapid increase in the number and availability of serological assays detecting SARS-CoV-2 antibodies, critical knowledge gaps remain regarding the magnitude and kinetics of the correlation between results of these assays and the presence of neutralizing antibodies.

Only a subset of antibodies against a specific antigen can neutralize viral replication. Assays that measure neutralizing antibody levels, such as plaque reduction neutralization tests (PRNT) and microneutralization methods, provide essential data, both for the validation of candidate diagnostic tests and to define serological correlates of immunity. These functional cell-based assays of SARS-CoV-2 neutralization can only be performed in a Biosafety Level 3 (BSL-3) laboratory, which is labour-intensive, costly, and severely limits testing throughput. Pseudotyped viruses have been developed that incorporate the Spike protein of SARS-CoV-2 and can be cultivated in BSL-2 conditions (3). Assays incorporating such pseudotyped viruses provide a functional assessment of the host neutralizing antibody responses as an alternative to using the wild-type virus (4-7). By contrast, surrogates of neutralization that bypass the need for viral culture would offer substantial advantages in terms of throughput, cost, and scalability. At least one direct ELISA assay detecting antibodies to the whole Spike protein has received regulatory approval in Europe for assessment of neutralizing antibodies (8). Further, several groups have proposed blocking assays, leveraging different signal detection methods to quantify the presence of host antibodies that can block the interaction of the SARS-CoV-2 Spike protein with human ACE-2 receptor (9-12).
On 6 Nov 2020, the FDA issued an emergency use authorization (EUA) for the cPass SARS-CoV-2 Neutralization Antibody Detection Kit (cPass; Genscript, Piscataway, NJ)(13), which is the first such surrogate neutralization assay to be commercially available. The cPass uses a blocking ELISA format with human ACE-2 receptor molecules coated on an ELISA plate (9, 14). Human sera pre-incubated with labelled epitopes of the receptor binding domain (RBD on S1 proteins) are then transferred to the plate. This blocking ELISA serves as a surrogate assay to inform on the capacity of human sera to block the interaction between the Spike fusion protein (through its RBD) and its cellular receptor ACE-2.

Thus, we aimed to inform the use of the cPass and assess its added value compared to laboratory-developed anti-RBD ELISA assays by performing an evaluation using a variety of well characterised specimens. A number of reference panels were utilized to allow an understanding of the ability of the cPass assay to detect significant titres of neutralizing antibodies assessed by culture-based reference methods. We compared cPass to PRNT and to a pseudotyped virus neutralization assay. We also sought to describe the correlation of cPass with a laboratory-developed indirect ELISA detecting anti-RBD IgG, IgM, and IgA antibodies at a single timepoint and across different timeframes among specimens collected at a known interval from onset of symptoms of SARS-CoV-2 infection.
METHODS

Ethics

All work was conducted in accordance with the Declaration of Helsinki in terms of informed consent and approval by an appropriate institutional board. Convalescent plasmas were obtained from donors who consented to participate in this research project at Héma-Québec, the agency responsible for blood supply in Quebec, Canada, (Research Ethics Board [REB] # 2020-004) and the Centre de Recherche du Centre Hospitalier de l’Université de Montréal (CR-CHUM) (REB # 19.381). The donors met all donor eligibility criteria: previous confirmed COVID-19 infection and complete resolution of symptoms for at least 14 days. At the Research Institute of the McGill University Health Centre (RI-MUHC), where cPass testing was performed, an REB exemption was granted on the basis that this work was considered to be a laboratory quality improvement project with no risk to participants.

Source of specimens tested

We assembled several well-characterised SARS-CoV-2 specimen panels to assess the performance characteristics of the cPass culture-free neutralization antibody detection kit (Table 1). These panels included: the Public Health Agency of Canada’s National Microbiology Laboratory SARS-CoV-2 panel of serological samples from COVID-19 patients, healthy individuals, as well as patients non-SARS-CoV-2 infections (Supplemental Table 1); the World Health Organization’s “First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin” (NIBSC code 20/268) (15); and two separate curated panels from Héma-Québec and CR-CHUM. The later panels comprised convalescent plasma donors with either longitudinal or single timepoint follow-up after SARS-CoV-2 diagnosis. Diagnosis of infection was either via nucleic acid amplification (NAAT), or by a case-definition requiring symptomatic disease and contact with a NAAT-confirmed individual.
Culture-free neutralization antibody detection assay (cPass)

All the specimens, including positive and negative controls provided with the kit, were processed according to the manufacturer’s instructions that included a 10X dilution factor of the primary specimen. To assure the validity of the results, all controls met the manufacturer’s requirements. All specimens and controls were tested in triplicate and the percentage of inhibition calculation was based on the mean of OD for each triplicate. A cut-off of 30% for SARS-CoV-2 neutralizing antibody detection was used to determine the presence of neutralizing antibodies, based on the manufacturer’s instructions for use. Kits were provided in kind by GenScript, but the manufacturer had no role in the design of the study, analysis of the data, or decision to submit the manuscript for publication.

Detection of neutralizing antibodies by culture-based reference methods

Neutralizing antibodies were detected via either assessment of plaque reduction neutralization titres using wild-type SARS-CoV-2, or by determining the neutralization half-maximal inhibitory dilution (PLV ID50) or the neutralization 80% inhibitory dilution (PLV ID80) of pseudotyped lentiviral vector (16).

Assessment of plaque-reduction neutralization using wild-type SARS-CoV-2 was performed at the Public Health Agency of Canada’s National Reference Laboratory for Microbiology. Briefly, serological specimens were diluted 2-fold from 1:20 to 1:640 in DMEM supplemented with 2% FBS and challenged with 50 plaque forming units (PFU) of SARS-CoV-2 (hCoV-19/Canada/ON_ON-VIDO-01-2/2020, EPI-ISL_425177), which were titrated by plaque assay (17). After 1 hour of incubation at 37°C and 5% CO2, the sera-virus mixtures were added to 12-well plates containing Vero E6 cells at 90% to 100% confluence and incubated at 37°C and 5% CO2 for 1 hour. After adsorption, a liquid overlay comprising 1.5% carboxymethylcellulose diluted in MEM supplemented with 4% FBS, L-glutamine,
non-essential amino acids, and sodium bicarbonate was added to each well and plates were incubated at 37°C and 5% CO2 for 72 hours. The liquid overlay was removed, and cells were fixed with 10% neutral-buffered formalin for 1 hour at room temperature. The monolayers were stained with 0.5% crystal violet for 10 minutes and washed with 20% ethanol. Plaques were enumerated and compared to controls. The highest serum dilution resulting in 50% and 90% reduction in plaques compared with controls were defined as the PRNT-50 and PRNT-90 endpoint titres, respectively. PRNT-50 titres and PRNT-90 titres ≥1:20 were considered positive for SARS-CoV-2 neutralizing antibodies.

Pseudoviral neutralization testing was performed as previously described (16).

Briefly, target cells were infected with single-round luciferase-expressing lentiviral particles. HEK 293T cells were transfected by the calcium phosphate method with the lentiviral vector pNL4.3 R-E- Luc (NIH AIDS Reagent Program) and a plasmid encoding for SARS-CoV-2 Spike at a ratio of 5:4. Two days post-transfection, cell supernatants were harvested and stored at −80°C until use. 293T-ACE2 target cells were seeded at a density of 1 x 10⁴ cells/well in 96-well luminometer-compatible tissue culture plates (Perkin Elmer) 24h before infection. Recombinant viruses in a final volume of 100 µL were incubated with the indicated sera dilutions (1/50; 1/250; 1/1250; 1/6250; 1/31250) for 1h at 37°C and were then added to the target cells followed by incubation for 48h at 37°C; cells were lysed by the addition of 30 µL of passive lysis buffer (Promega) followed by one freeze-thaw cycle. An LB942 TriStar luminometer (Berthold Technologies) was used to measure the luciferase activity of each well after the addition of 100 µL of luciferin buffer (15mM MgSO₄, 15mM KPO₄ [pH 7.8], 1mM ATP, and 1mM dithiothreitol) and 50 µL of 1mM d-luciferin potassium salt (ThermoFisher Scientific). The neutralization half-maximal inhibitory dilution (ID₅₀) or the neutralization 80% inhibitory dilution (ID₈₀) represents the sera dilution to inhibit 50% or
80% of the infection of 293T-ACE2 cells by recombinant viruses bearing the indicated surface glycoproteins.

**Indirect antiRBD ELISA assays**

Specimens were analysed with a laboratory-developed indirect ELISA detecting anti-RBD IgG, IgM, and IgA as previously described (16).

**Statistical analysis**

The diagnostic accuracy of the cPass surrogate viral neutralization assay was estimated compared to different reference standards (WT PRNT-50; WT PRNT-90; PLV ID50; PLV ID80, Live Virus (CPE), and VSV-PV). Sensitivities and specificities are presented with 95% confidence intervals (95% CI). The effect of varying the cut-off value (i.e., % inhibition of RBD-ACE2 binding) for cPass positivity on the diagnostic accuracy of the cPass against a PLV PRNT-50 reference standard was investigated using a receiver operating characteristic (ROC) curve. The association between cPass % inhibition and results obtained using laboratory-developed ELISA detecting anti-S-RBD IgG, IgM, and IgA are presented in scatterplots with the strength of these associations informed by Pearson correlation. Lastly, among specimens with a known interval from onset of SARS-CoV-2 infection symptoms (n=79), spaghetti plots were created to investigate any change in signal over time for the cPass and direct anti-S-RBD ELISA with statistical significance assessed using the Wilcoxon matched-pairs signed-rank test (p<0.05 denoted by *). Statistical analyses were performed using R version 3.5.2 (R Core Team, Vienna, Austria).

**RESULTS**
Diagnostic accuracy for the detection of anti-SARS-CoV-2 neutralizing antibodies, and the impact of using different reference standards

Table 1 shows the estimated diagnostic accuracy of the GenScript cPass neutralization antibody detection assay among well characterised specimen panels, according to different reference standards. Among various reference standards, results from the same PLV ID50 assay were available for all panels except the WHO panel, and this was used to estimate aggregate diagnostic accuracy values across several panels.

Overall, cPass had very high sensitivity and specificity compared to the reference standard of a 50% plaque reduction neutralization using SARS-CoV-2 viral culture (WT PRNT-50). This remained the case whether a cut-off titre of 1:20 or 1:50 was used [sensitivity 100% (95%CI 82-100) for both cut-offs, specificity 95% (95%CI 76-100) and 91% (95%CI 71-99), respectively]. Sensitivity remained very high compared to the reference standard of a neutralization half-maximal inhibitory dilution using a validated pseudotyped lentiviral vector neutralization assay (PLV ID50) with a cut-off titre of 1:50, but specificity was lower than that compared to WT PRNT-50, ranging from 17-70% (Table 1).

The effect of cut-off values on the diagnostic accuracy of the GenScript cPass assay is shown in Figure 1. A receiver operating characteristic (ROC) curve using the reference standard of PLV ID50 yielded an area under the ROC curve of 0.802.

Effect of serial dilution on the accuracy for detecting sera with positive PRNT-90 titres

Against the most stringent reference standard of 90% plaque reduction neutralization using SARS-CoV-2 viral culture (WT PRNT-90), estimated specificity was reduced compared to WT PRNT-50. Specificity remained similar whether a cut-off WT PRNT-90 titre for positivity of 1:20 or 1:50 was used [61% (95%CI 42-77) and 57% (95%CI 39-74),
respectively] (Table 1). We performed serial dilution of the 16 primary specimens from the
National Microbiology Laboratory Panel with WT PRNT-50 titres ≥1:20 to determine
whether we could establish a dilution that increased specificity for detecting those with WT
PRNT-90 titres ≥1:20 without sacrificing sensitivity. A 50-fold dilution of specimens with
positive WT PRNT-50 titres increased specificity for those with positive WT PRNT-90 titres
from 11% (95%CI 0-48) to 100% (95%CI 66-100), with one missed PRNT-90 positive
specimen. Results are summarised in Figure 2.

Correlation of the GenScript cPass assay with anti-RBD ELISA, and signal variation
according to time interval since onset of SARS-CoV-2 infection symptoms.

Results obtained with cPass were compared to those obtained using laboratory-
developed ELISA detecting anti-RBD IgG, IgM, and IgA to assess whether the cPass yields
complementary information (Figure 3). Highest agreement between cPass percent inhibition
of RBD-ACE2 binding and ELISA area under the curve (AUC) was seen for anti-RBD IgG
(r=0.851 at 0-6 weeks; r=0.798 at > 6 weeks). The diagnostic accuracy of categorical anti-
RBD IgG results for the detection of SARS-CoV-2 neutralizing antibodies was essentially
identical to that observed with the cPass for all panels and reference standards (Table 2).

Among paired specimens from the same individual collected at a known interval from
SARS-CoV-2 diagnosis, aggregate results of both cPass and direct anti-RBD IgG ELISA did
not change between 6 weeks and 10 weeks after diagnosis (p=1.00 and 0.104, respectively,
by the Wilcoxon signed rank test) (Figure 4). In contrast, optical densities decreased
significantly over the same timeframe for direct anti-RBD IgM (p=0.0058) and IgA
(p=0.0012) ELISA.
DISCUSSION

Rapid and high throughput surrogates for PRNT or pseudovirus neutralization assays that bypass the need for cell culture are awaited with the belief that they will offer additional information to that from standard direct immunoassays, such as a higher specificity for neutralizing antibodies. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit (cPass) is the first such assay to be commercially available and to receive FDA EUA in the U.S. An evaluation of a cPass prototype, using a cut-off value of 20% inhibition, found that it could provide a high-throughput screening tool for confirmatory PRNT testing (18). The results of the current evaluation support the ability for cPass to detect neutralizing antibodies to SARS-CoV-2, and extend our understanding of how cPass results compare to those obtained with non-blocking anti-RBD ELISA among varied well characterised specimen panels.

The estimated sensitivity of cPass for detection of anti-SARS-CoV-2 neutralizing antibodies was consistently very high, regardless of the reference standard technique or cut-off titre for positivity. Despite the fact that several groups have described anti-SARS-CoV-2 neutralizing antibodies that target non-RBD epitopes (19-21), our results do not suggest that this assay targeting only RBD-ACE2 blockade would miss a substantial proportion of patients with neutralizing antibodies identified by a functional cell-culture-based reference standard. This may be the case because neutralizing antibodies to non-RBD epitopes usually occur concomitantly with anti-RBD neutralizing antibodies, instead of in isolation (19-21). This requires elucidation.

By contrast, estimates of the specificity of cPass for the detection of anti-SARS-CoV-2 neutralizing antibodies were highly contingent of the reference standard used. There was near-perfect negative agreement with WT PRNT-50 using a cut-off titre of either 1:20 or 1:50
[95% (95%CI 76-100) and 91% (95%CI 71-99), respectively]. However, negative agreement
was much lower when cPass was compared to either PLV ID50 or WT PRNT-90 (Table 1).
Our data raise the unresolved question of which reference technique (i.e., wild-type or
pseudotyped live viral culture), level of stringency (e.g., 50% inhibition of infection vs 80%,
90%, etc), and cut-off titre (e.g., 1:20 vs 1:50) best represent serocorrelates of protection to
SARS-CoV-2, or other relevant applications such as the screening of sera for use in
convalescent plasma trials. Moreover, protocols can vary widely for the same technique
across different laboratories, requiring caution in the interpretation of these and other data
(22). In the current manuscript, PLV ID50 with a cut-off titre of 1:50 was used as the overall
comparator because it was the technique applied to all available specimen panels. Our results
must be interpreted in context with this potential source of bias. However, we note that this
technique has been widely employed by other groups and as such offers a high degree of
generalizability with other results (23, 24).

The cPass assay detected all specimens with positive WT PRNT-90 titres, with a
significant proportion of false positives (Figure 2). This suggests that, within the panel tested,
the functional assessment of RBD-ACE2 blocking antibodies did not miss specimens that
may have included neutralizing antibodies to other targets in addition to those against RBD.
A 50-fold dilution of the 16 primary specimens with WT PRNT-50 titres ≥1:20 increased
specificity for detecting those with WT PRNT-90 titres ≥1:20 from 11% (95% CI 0-48) to
100% (95% CI 66-100). This may represent a useful approach for using the cPass assay to
identify blood specimens with positive WT PRNT-90 titres, which has been proposed as a
desirable characteristic for sera used in convalescent plasma trials by some regulatory
agencies.
Finally, results of the cPass assay are best correlated with those of a laboratory-developed indirect anti-RBD ELISA detecting IgG, both at a single timepoint (Table 2, Figure 3) and across time among paired specimens form the same individual collected at a known interval from symptoms onset (Figure 4). The fact that results of cPass and anti-RBD IgG remained stable between 6 and 10 weeks post-symptom onset, while optical densities decreased significantly over the same timeframe for anti-RBD IgM and IgA ELISA is potentially concerning given recent work suggesting a major role of IgM and IgA in the neutralizing activity of convalescent plasma against SARS-CoV-2 (25-28). The observed trend toward lower specificity at later timepoints among convalescent plasma donors with longitudinal follow-up (i.e. [60% (95% CI 15-95)] at 6 weeks vs [17% (95% CI 0-64)] at 10 weeks) may thus be related to loss of neutralizing IgM (Table 1). In addition, specificity of the cPass may be affected by the possibility that part of the inhibition of binding in the cPass assay could be due to steric hindrance by the abundant anti-Spike antibodies of the IgG isotype rather than by true neutralization (as occurs in vivo).

**CONCLUSIONS**

The results of the current evaluation demonstrate the ability of cPass to detect blood specimens with anti-SARS-CoV-2 neutralizing antibodies, but they do not support a clear added value of cPass compared to a laboratory-developed indirect anti-RBD ELISA detecting IgG antibodies. Whether this is also the case for commercially-available direct anti-RBD ELISAs deserves further investigation.
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**TABLE 1.** Diagnostic accuracy of the GenScript cPass surrogate viral neutralization assay to detect neutralizing antibodies among well-characterised specimen panels, according to reference standard used

| SOURCE | Number | Reference standard | Cut-off for reference positivity<sup>a</sup> | TP | FP | FN | TN | Sensitivity % (95% CI) | Specificity % (95% CI) |
|--------|--------|--------------------|--------------------------------------------|----|----|----|----|------------------------|------------------------|
| National Microbiology Laboratory panel (Canada) | 16 SARS-CoV-2 PCR positive 24 negative for SARS-CoV-2 and positive for related infections | WT PRNT-50 | 1:20 | 19 | 1 | 0 | 20 | 100 (82-100) | 95 (76-100) |
| | | | 1:50 | 18 | 2 | 0 | 20 | 100 (81-100) | 91 (71-99) |
| | | WT PRNT-90 | 1:20 | 7 | 13 | 0 | 20 | 100 (59-100) | 61 (42-77) |
| | | | 1:50 | 5 | 15 | 0 | 20 | 100 (48-100) | 57 (39-74) |
| | | PLV ID50 | 1:50 | 12 | 8 | 1 | 19 | 92 (64-100) | 70 (50-86) |
| | | PLV ID80 | 1:50 | 10 | 10 | 0 | 20 | 100 (69-100) | 67 (47-83) |
| WHO panel (UK) | 3 SARS-CoV-2 positive 2 SARS-CoV-2 negative | WT PRNT-50 | 1:20 | 2 | 1 | 0 | 2 | 100 (16-100) | 67 (9-99) |
| | | Live Virus (CPE) | 1:20 | 3 | 0 | 1 | 1 | 75 (19-99) | 100 (3-100) |
| | | VSV-PV | 1:20 | 3 | 0 | 0 | 2 | 100 (29-100) | 100 (16-100) |
| Blood bank - convalescent plasma donors with longitudinal follow-up<sup>b</sup> | Specimens characterised by anti-S-RBD ELISA and PLV ID50 | PLV ID50 | 1:50 | 10 | 2 | 0 | 3 | 100 (69-100) | 60 (15-95) |
| | | Specimens characterised by anti-S-RBD ELISA and PLV ID50 | PLV ID50 | 1:50 | 8 | 5 | 0 | 1 | 100 (63-100) | 17 (0-64) |
| Specimens characterised by anti-S-RBD ELISA and PLV ID50 | PLV ID50 | 1:50 | 24  | 12  | 4   | 10  | 86 (67-96) | 45 (24-68) |
|---------------------------------------------------------|----------|------|-----|-----|-----|-----|------------|------------|
| Blood bank-convalescent plasma donors with single timepoint follow-up |          |      |     |     |     |     |            |            |
| 0-6 weeks post-symptom onset                              |          | 11  | 6  | 1  | 0  |     | 92 (62-100) | 0 (0-46)   |
| >6 weeks post-symptom onset                               |          | 13  | 6  | 3  | 10 |     | 81 (54-96) | 62 (35-85) |
| Overall (vs PLV ID50)                                    |          | 54  | 27 | 5  | 33 |     | 92 (81-97) | 55 (42-68) |

\(^a\) Cut-off used to determine cPass positivity was ≥30%.

\(^b\) From patients meeting public health case definitions of COVID-19, with either NAAT-confirmed SARS CoV-2 infection or an epidemiological link to a known case of COVID-19 (SARS CoV-2 infection).

\(^c\) Results from the same PLV ID50 assay were available for all panels except the WHO panel; PLV ID50 assay was used to calculate overall diagnostic accuracy values.

WT PRNT-50 or PRNT-90 denotes neutralization titres required for a 50% or 90% plaque reduction, respectively, using SARS-CoV-2 viral culture; PLV ID50 or PLV ID80 denotes the serum dilution to inhibit 50% or 80% of the infection of 293T-ACE2 cells by recombinant viruses bearing the indicated surface glycoproteins; TP true positive; FP false positive; FN false negative; TN true negative; Anti-S-RBD antibodies against receptor binding domain of SARS-CoV-2 Spike protein; HQ Héma-Québec; WHO World Health Organization; VSV PV Vesicular stomatitis virus pseudovirus; CPE cytopathic effect; NAAT nucleic acid amplification test.
474 **TABLE 2.** Diagnostic accuracy of a laboratory-developed IgG anti-RBD ELISA to detect neutralizing antibodies

| SOURCE | Number | Reference standard | Cut-off for positivity\(^a\) | TP | FP | FN | TN | Sensitivity | Specificity |
|--------|--------|--------------------|-----------------------------|----|----|----|----|-------------|-------------|
| National Microbiology Laboratory panel (Canada) | 16 SARS-CoV-2 positive | WT PRNT-50 | 1:20 | 19 | 1 | 0 | 20 | 100 (82-100) | 95 (76-100) |
| | 24 negative for SARS-CoV-2 and positive for related infections | | | | | | | | |
| | 1:50 | 18 | 2 | 0 | 20 | 100 (81-100) | 91 (71-99) |
| PLV PRNT-50 | 1:50 | 12 | 8 | 1 | 19 | 92 (64-100) | 70 (50-86) |

\(^a\)Cut-off used to determine cPass positivity was ≥30%.

WT PRNT-50 or PRNT-90 denotes neutralization titres required for a 50% or 90% plaque reduction, respectively, using SARS-CoV-2 viral culture; TP true positive; FP false positive; FN false negative; TN true negative; Anti-S-RBD antibodies against receptor binding domain of SARS-CoV-2 Spike protein; HQ Héma-Québec; WHO World Health Organization; VSV PV Vesicular stomatitis virus pseudovirus; CPE cytopathic effect.
**FIGURE LEGENDS**

**Figure 1.** Effect of cut-off values on the diagnostic accuracy of the Genscript cPass SARS-CoV-2 neutralization antibody detection kit. Panel (A) shows the receiver operating characteristic (ROC) curve, with different cPass cutoffs. Panel (B) details results and estimates of sensitivity and specificity for different %inhibition of RBD-ACE2 binding cutoffs for cPass positivity. The reference standard used is PLV PRNT 50 at a titre of ≥1:50. AUC denotes Area Under the ROC Curve; TP true positive; FP false positive; FN false negative; TN true negative.

**Figure 2.** Effect of serial dilution on the accuracy for detecting sera with positive PRNT90 titres. Serial dilution of the 16 primary specimens with WT PRNT 50 titres ≥1:20 was performed to establish a dilution that increased specificity for detecting those with WT PRNT 90 titres ≥1:20. Panel (A) shows individual data points according to dilution and WT PRNT 90 status (positive ≥1:20). Box plots depict the median and interquartile range. Panel (B) details results and estimates of sensitivity and specificity for serial dilution factor. All dilution factors are additional to the 10X dilution required in the manufacturer’s instructions. WT PRNT 90 denotes neutralization titres required for a 90% plaque reduction using SARS-CoV-2 viral culture; TP true positive; FP false positive; FN false negative; TN true negative.

**Figure 3.** Correlation of the Genscript cPass assay with anti-S-RBD ELISA. Scatterplots and Pearson correlation coefficient for results obtained with cPass compared to those obtained using laboratory-developed ELISA detecting anti-RBD IgG, IgM, and IgA (Panels A, B, C, respectively). The vertical dashed line depicts the manufacturer’s recommended cut-off for cPass positivity.
Figure 4. Change of signal over time for Genscript cPass and anti-RBD ELISA.

Spaghetti plot of results obtained with cPass (panel A) and optical densities of laboratory-developed ELISA detecting anti-RBD IgG, IgM, and IgA (panels B, C, D, respectively) among specimens collected at a known interval from SARS-CoV-2 diagnosis. Horizontal lines indicate paired specimens form the same individual. P values are calculated via the Wilcoxon signed rank test, and values <0.05 are designated with an Asterix. In all panels, red dots denote specimens with positive cPass results, and blue dots specimens with negative cPass results.
Figure 1. Effect of cut-off values on the diagnostic accuracy of the Genscript cPass SARS-CoV-2 neutralization antibody detection kit.

Panel (A) shows the receiver operating characteristic (ROC) curve, with different cPass cutoffs. Panel (B) details results and estimates of sensitivity and specificity for different %inhibition of RBD-ACE2 binding cutoffs for cPass positivity. The reference standard used is PLV PRNT 50 at a titre of ≥1:50. AUC denotes Area Under the ROC Curve; TP true positive; FP false positive; FN false negative; TN true negative.
Figure 2. Effect of serial dilution on the accuracy for detecting sera with positive PRNT90 titres.

Serial dilution of the 16 primary specimens with WT PRNT 50 titres ≥1:20 was performed to establish a dilution that increased specificity for detecting those with WT PRNT 90 titres ≥1:20. Panel (A) shows individual data points according to dilution and WT PRNT 90 status (positive ≥1:20). Box plots depict the median and interquartile range. Panel (B) details results and estimates of sensitivity and specificity for serial dilution factor. All dilution factors are additional to the 10X dilution required in the manufacturer’s instructions. WT PRNT 90 denotes neutralization titres required for a 90% plaque reduction using SARS-CoV-2 viral culture; TP true positive; FP false positive; FN false negative; TN true negative.
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### Table

| Dilution Factor | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) |
|-----------------|----|----|----|----|----------------------|----------------------|
| 1X               | 7  | 8  | 0  | 1  | 100 (59-100)         | 11 (0-48)            |
| 10X              | 7  | 3  | 0  | 6  | 100 (59-100)         | 67 (30-93)           |
| 50X              | 6  | 0  | 1  | 9  | 86 (42-100)          | 100 (66-100)         |
| 100X             | 4  | 0  | 3  | 9  | 57 (18-90)           | 100 (66-100)         |

### Figure A

Box plots showing the percent inhibition for different dilution factors for primary specimens. The PRNT90 result is indicated with blue dots for negative and red dots for positive outcomes.

### Figure B

A table showing the counts of true positives (TP), false positives (FP), false negatives (FN), true negatives (TN), sensitivity, and specificity for different dilution factors.
