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Abstract

The true severity of infection due to COVID-19 is under-represented because it is based on only those who are tested. Although nucleic acid amplifications tests (NAAT) are the gold standard for COVID-19 diagnostic testing, serological assays provide better population-level SARS-CoV-2 prevalence estimates. Implementing large sero-surveys present several logistical challenges within Canada due its unique geography including rural and remote communities. Dried blood spot (DBS) sampling is a practical solution but comparative performance data on SARS-CoV-2 serological tests using DBS is currently lacking. Here we present test performance data from a well-characterized SARS-CoV-2 DBS panel sent to laboratories across Canada representing 10 commercial and 2 in-house developed tests for SARS-CoV-2 antibodies. Three commercial assays identified all positive and negative DBS correctly corresponding to a sensitivity, specificity, positive predictive value, and negative predictive value of 100% (95% CI = 72.2, 100). Two in-house assays also performed equally well. In contrast, several commercial assays could not achieve a sensitivity greater than 40% or a negative predictive value greater than 60%. Our findings represent the foundation for future validation studies on DBS specimens that will play a central role in strengthening Canada's public health policy in response to COVID-19.

Dried blood spot specimens for SARS-CoV-2 antibody testing: A multi-site, multi-assay comparison

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Introduction

According to the latest estimates from John Hopkins University (last accessed on 26 March 2021), severe acute respiratory syndrome coronavirus (SARS-CoV2), the etiological agent of coronavirus disease 2019 (COVID-19)¹, is responsible for over 125 million confirmed cases, including over 2.7 million deaths globally². While nucleic acid amplification tests (NAAT) on respiratory samples remain the gold standard for COVID-19 diagnostic testing³, they cannot provide reliable prevalence and incidence estimates at the population level given the narrow window for reliable results (<14 days post-symptom onset)⁴, global supply shortages⁵, and limited access due to symptom-based testing prioritization⁶. On the other hand, serological assays may provide more reliable population-level SARS-CoV-2 prevalence estimates to better inform ongoing public health responses⁷. Serological testing is also vitally important for monitoring both individual and population-level humoral immune responses to COVID-19 vaccination⁸.

Several commercial serological assays rapidly became available through measures such as the US Food and Drug Administration's (FDA) Emergency Use Authorization programme⁹. These assays have been primarily designed for the detection of SARS-CoV-2 antibodies in serum, plasma, or whole blood¹⁰⁻¹³. Implementing large-scale integrated biological-behavioural surveys poses a significant challenge since phlebotomy requires highly trained personnel when most health care professionals have been re-deployed to assist in the COVID-19 response¹⁴. Furthermore, reaching rural and remote communities adds to the complexity of providing reliable and timely testing for several reasons including lack of trained personnel to collect biological specimens, limited access to laboratory facilities, difficulties in maintaining the cold chain, and unreliable

specimen transportation even within the Canadian context. The implementation of SARS-CoV-2 point-of-care (POC) testing¹⁵⁻¹⁸ could alleviate some of these challenges but may not be appropriate in all settings. Rural and remote communities tend to be small and have close-knit social networks thereby making confidential or anonymous SARS-CoV-2 POC testing difficult. Thus, a practical solution is required to be able to achieve large scale sampling and circumvent these problematic issues. Dried blood spot (DBS) collection may be a practical solution owing to this method's simplicity.

DBS are prepared by placing a few drops of blood on a card made of filter paper. A finger prick is performed using standard spring-loaded lancets that do not require specialised training to utilize. Home-self collection is also a viable option that is already playing a significant role in SARS-CoV-2 sero-surveillance^{19,20}. Once the DBS cards are dry, they can be stored and transported at ambient temperature to centralized laboratories through regular mail services without any adverse effect on downstream testing²¹. However, comparative performance data on SARS-CoV-2 serological assays using DBS specimens are limited. Here we present test performance data from a SARS-CoV-2 DBS panel sent to various public health and academic laboratories across Canada representing 10 commercial and 2 in-house developed assays for SARS-CoV-2 antibodies. As part of the COVID-19 Immunity Task Force (CITF, www.covid19immunitytaskforce.ca/), our goal was to provide preliminary performance data on each test to guide future large-scale validations.

Results

Assay performance. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were assessed for 10 commercial and 2 in-house assays on 10 known negative plasma samples and 10 plasma samples from COVID-19 patients (Table 3) contrived as DBS. Nearly one third of all commercial assays (EUROIMMUN, Elecsys spike, and GSP/DELFIA) identified all positive and negative DBS correctly corresponding to a sensitivity, specificity, PPV, and NPV of 100% (95% CI = 72.2, 100). The Platelia assay achieved a sensitivity, specificity, PPV, and NPV of 100% (95% CI = 72.2, 100), 90% (95% CI = 59.6, 99.5), 90.9% (95% CI = 62.3, 99.5), and 100% (95% CI = 70.1, 100) respectively due to one equivocal result from a negative DBS sample. The Elecsys nucleocapsid assay achieved a sensitivity, specificity, PPV, and NPV of 90% (95% CI = 59.6, 99.5), 100% (95% CI = 72.2, 100), 100% (95% CI = 70.1, 100), and 90.9% (95% CI = 62.3, 99.5) respectively after increasing the sample input from 2 to 4 DBS punches. Performance varied for the remaining commercial assays. The LIASON, Architect, and COV2G assays achieved a specificity and PPV of 100% (95% CI = 72.2, 100) and 100% (95% CI = 5.1, 100) respectively but could not achieve a sensitivity greater than 20% (95% CI = 3.6, 51) or an NPV greater than 55.6% (95% CI = 33.7, 75.4) despite increasing the sample input from 2 to 4 DBS punches. The COV2T assay achieved a sensitivity, specificity, PPV, and NPV of 40% (95% CI = 16.8, 68.7), 60% (95% CI = 31.3, 83.2), 50% (95% CI = 21.5, 78.5), and 50% (95% CI = 25.4, 74.6) respectively after increasing the sample input from 2 to 4 DBS punches. Similarly, the VITROS assay achieved a sensitivity and PPV of 100% (95% CI = 72.2, 100) and 50% (95% CI = 29.9, 70.1) respectively but could not achieve a specificity greater than 0% despite increasing sample input.

The in-house assays from the University of Ottawa and University of Toronto performed well and successfully identified all positive and negative DBS correctly corresponding to a sensitivity, specificity, PPV, and NPV of 100% (95% CI = 72.2, 100). However, the in-house nucleocapsid (U of T) assay test only achieved a sensitivity, specificity, PPV, and NPV of 40% (95% CI = 16.8, 68.7), 100% (95% CI = 72.2, 100), 100% (95% CI = 51, 100), and 62.5% (95% CI = 38.6, 81.5) respectively.

Separation between positive and negative samples. Raw data values were plotted, and receiver operating characteristic (ROC) curves were computed (Figure 1-2, Supplementary Figure 2-3, Table 4) to evaluate each assay's ability to separate the 10 known negative plasma samples and 10 plasma samples from COVID-19 patients contrived as DBS. Among the commercial assays, the EUROIMMUN, Platelia, Elecsys spike, Elecsys nucleocapsid, and Architect showed the clearest separation of positive and negative samples by DBS testing. On the other hand, the LIASON, COV2G, and COV2T could not clearly distinguish between positive and negative DBS specimens. The VITROS assay showed good separation of positive and negative DBS and the ROC curve suggests that increasing the signal to cut-off (S/Co) ratio from 1.0 to 3.4 could potentially increase this assay's sensitivity and specificity to 90% (95% CI = 60, 99) and 100% (95% CI = 72.2, 100) respectively. The LIASON, COV2G, and COV2T could not clearly differentiate positive and negative specimens by DBS testing.

The in-house assays from the University of Ottawa and University of Toronto (spike, RBD, and nucleocapsid) showed a clear separation between positive and negative DBS (Figure 1-2, Supplementary Figure 2-3, Table 4). However, the in-house

nucleocapsid (University of Toronto) assay was unable to differentiate all positive and negative DBS successfully.

Discussion

On a small panel of DBS, this study has shown that the EUROIMMUN, Elecsys spike, and GSP/DELFI commercial assays as well in-house assays are capable of achieving a sensitivity, specificity, PPV, and NPV of 100% (95% CI = 72.2, 100) following testing of DBS eluate samples collected ≥ 36 days post symptom onset without optimization. We interpret these findings cautiously since a recent systematic review by Lisboa Bastos *et al.*²² assessing 40 serological studies reported that the maximum pooled sensitivities for enzyme linked immunosorbent assays measuring IgG or IgM (ELISAs) and chemiluminescent immunoassays (CLIAs) was 84.3% (95% CI = 75.6, 90.9) and 97.8% (95% CI = 46.2, 100) respectively. The accuracy of serological assays also varies according to sampling time frames and sensitivity typically exceeds 90% between 15-35 days post symptom onset²². We were unable to stratify our analysis according to days post symptom onset due to our sample size and narrow range of collection dates. Furthermore, our samples consisted of COVID-19 convalescent plasma donors who are likely to have significant antibody titers²³. Nonetheless, we clearly show that certain assays like the LIASON, COV2G, and COV2T could not reliably identify positive and negative specimens by DBS testing.

Based on our ROC curve analysis, assays like the Platelia, Elecsys nucleocapsid, VITROS, and Architect, which did not reach 100% for all performance characteristics, could clearly distinguish between positive and negative samples therefore could also

potentially achieve a sensitivity, specificity, PPV, and NPV near 100% via threshold adjustments²⁴⁻²⁶. However, establishing appropriate thresholds will require a study using a large number of well characterised clinical samples representing a broad range of factors associated with developing robust immune responses to SARS-CoV-2 such as sex, age, immunodeficiencies, and disease severity²⁷.

This validation study has several limitations that must be considered. First, our panel was small, albeit well characterized. Second, we did not take into consideration other capillary blood collection methods such as capillary tubes. While capillary tubes have been shown to be a practical alternative to venepuncture for SARS-CoV-2 antibody testing^{28,29}, they cannot be transported as easily or safely as DBS therefore their applicability are somewhat limited for large sero-surveys within the Canadian context. Last, DBS eluates, prepared using a well-characterized buffer used specifically by the NLHRS, were sent to the participants (with the exception of the PE GSP-Delphia). The assessment of different buffers or the further optimization of DBS elution conditions was beyond the scope of this study. Additional refinement of these procedures may have yielded more optimistic results on the assays that were not reported to fare well in this evaluation. However, the purpose of this evaluation was to develop a simplified procedure based on simple well-characterized practices previously developed within the NLHRS. Implementing this standardized approach allowed for an effective and timely identification of promising platforms that could be used for DBS testing for anti-SARS-CoV-2 antibodies without extensive modification. We believe that this was accomplished despite these limitations.

In conclusion, we assessed the performance of 10 commercial and 2 in-house serological assays for DBS eluate testing. Several of these assays achieved a specificity, sensitivity, PPV, and NPV adequate for sero-surveys even in low-prevalence settings. These findings suggest that the high demand for SARS-CoV-2 serology testing, mainly driven by sero-surveys within the Canadian context, could be met by the collection and testing of DBS by several different assays, thereby minimizing the risk of shortages. Furthermore, DBS collection has the potential to expand testing use and access while limiting the requirements for specimen collection on healthcare professionals already overtaxed with the COVID-19 response. While our sample size was small, this validation study, undertaken initially to determine the feasibility of a nationally representative household-based sero-survey that would be based on DBS, resulted in the selection of the in-house assays. This was not only because of their test performance but also because they are capable of distinguishing infection-acquired from vaccine-induced antibodies to inform public health strategies in diverse jurisdictions across Canada. This validation study therefore represents the foundation for future validation studies on DBS specimens, that will undoubtedly play a central role in shaping Canada's public health policy in response to COVID-19.

Methods

Patient specimens. SARS-CoV-2 antibody positive and negative plasma used to contrive DBS specimens are described in Table 1. SARS-CoV-2 antibody positive plasma was collected from COVID-19 convalescent donors at Mount Sinai Hospital (Toronto, Canada). Plasma was tested for SARS-CoV-2 antibodies using the Platelia SARS-CoV-2 Total Ab (Bio-Rad, Hercules, California) or Anti-SARS-CoV-2 ELISA IgG

(EUROIMMUN, Lübeck, Germany) kits. SARS-CoV-2 negative plasma was collected from healthy donors within the National Microbiology Laboratory (Winnipeg, Canada). To prepare plasma, blood was collected in EDTA Vacutainer tubes (Beckton Dickinson, Franklin Lates, NJ) and centrifuged at 1,500 RPM for 7 minutes. Plasma was tested using the Platelia SARS-CoV-2 Total Ab (Bio-Rad) or Anti-SARS-CoV-2 ELISA IgG (EUROIMMUN, Lübeck, Germany) kits to verify donors were negative for SARS-CoV-2 antibodies. All assays were performed according to the manufacturer's instructions.

Contrived dried blood spot specimens. A panel consisting of 10 unique SARS-CoV-2 antibody positive and 10 unique SARS-CoV-2 negative DBS cards were contrived to assess the performance of commercial and in-house serological tests (Supplementary Figure 1). Each testing site was blinded to the status of the DBS cards. SARS-CoV-2 antibody positive plasma samples were contrived into DBS specimens by using blood collected from healthy donors within the National Microbiology Laboratory. SARS-CoV-2 antibody negative blood was centrifuged at 1,500 RPM for 7 minutes and the plasma was removed. The remaining red blood cells were re-suspended with SARS-CoV-2 antibody positive plasma using a 1:1 ratio and 75 µL was spotted onto each circle of a Whatman 903 Proteinsaver card (GE Healthcare, Boston, MA). Spotted cards were allowed to air-dry for at least 2 hours in a biosafety cabinet and then packaged in a gas impermeable bag with a desiccant pack and a humidity indicator card. Packaged cards (maximum 10 per bag) were stored at -80°C until further testing. SARS-CoV-2 antibody negative blood was spotted directly from the EDTA Vacutainer tubes onto Whatman 903 Proteinsaver cards as described above.

Dried blood spot elution. DBS samples were punched using a 6 mm hole punch into a 96 deep well plate. One to four 6 mm (1/4 inch) punches were added to each well and eluted in DPBS buffer (pH 7.4) containing 0.5% BSA and 0.05% Tween-20 overnight at 4°C with agitation (400 RPM). For the Anti-SARS-CoV-2 ELISA IgG (EUROIMMUN) a single punch was eluted in 500 µL of Sample Buffer overnight without agitation and 100 µL of eluate was transferred to the assay plate. For Platelia SARS-CoV-2 Total Ab (Bio-Rad) assay, two punches were eluted in 130 µL of Sample Diluent overnight with gentle agitation (400 RPM), 75 µL of eluate was mixed with conjugate, and 100 µL of the eluate-conjugate mixture was transferred to the assay plate. Elution volumes were adjusted for all other assays according to manufacturer's specifications or in-house developed protocols (Table 2). Afterwards, plates were incubated at room temperature for 30 minutes with agitation (400 RPM) and each DBS eluate was transferred to an individual 2.0 mL screw cap tube. Eluates were stored at -80°C until shipment on dry ice to each testing site.

SARS-CoV-2 antibody testing. DBS eluates were tested for SARS-CoV-2 antibodies with 10 commercial assays and 2 in-house assays (Table 2) according to the manufacturer's instructions or laboratory developed protocols respectively. No attempts were made at this point to optimize protocols for DBS specimens. The commercial tests consisted of 6 IgG based assays: Anti-SARS-CoV-2 ELISA (EUROIMMUN), LIASON SARS-CoV-2 (DiaSorin, Saluggia, Italy), SARS-CoV-2 COV2G (Siemens, Erlangen, Germany), Architect SARS-CoV-2 (Abbott, Mississauga, Canada), and GSP/DELFLIA Anti-SARS-CoV-2 (PerkinElmer, Waltham, Massachusetts); and 5 total antibody assays: Platelia SARS-CoV-2 (Bio-Rad), SARS-CoV-2 COV2T (Siemens), Elecsys quantitative

Anti-SARS-CoV-2 (Elecsys spike; Roche, Basel, Switzerland), Elecsys Anti-SARS-CoV-2 (Elecsys nucleocapsid; Roche), and VITROS Anti-SARS-CoV-2 (Ortho Clinical Diagnostics, Raritan, New Jersey). EUROIMMUN was included since it is the only assay currently approved by Health Canada for use with serum and DBS. Both in-house tests, described in greater detail elsewhere³⁰ and Table 2, consist of IgG assays targeting SARS-CoV-2 spike, receptor binding domain (RBD), and nucleocapsid proteins. Each testing site was responsible for interpreting and reporting their own data.

Statistical analysis. Statistical analysis was conducted using Prism version 9.0.0 (GraphPad Software, San Diego, CA). Assay performance on DBS expressed in terms of sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), was computed in comparison with the serum-based Anti-SARS-CoV-2 ELISA (EUROIMMUN) assay results as the gold standard. Confidence intervals were computed using the hybrid Wilson/Brown method³¹. Receiver operating characteristics (ROC) curves were also computed using the Wilson/Brown method. A P-value ≤ 0.05 was considered statistically significant.

Data availability. All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

Ethics statement. Ethical approval was obtained from the Health Canada and Public Health Agency of Canada Research Ethics Board (REB 2020-022P).

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Author contributions

C.M., P.L., and J.K. conceived and designed the experiments. A.H., H.E., and K.C. prepared the DBS panel, performed testing, and collected data. F.C., C.M., and J.K. conceived and performed the data analysis. F.C., C.M., P.L., and J.K. wrote, reviewed, and finalized the manuscript. Y.G., M.-A.L., A.-C.G., C.P.Y., J.P., M.P.C, P.C., D.S., P.V.C., S.B., M.K., D.G., A.M., C.O., and M.D. performed testing, provided data, and reviewed the manuscript. S.B. provided a figure for the supplementary information. B.M. and C.H. reviewed and assisted in finalizing the manuscript.

Competing interests

All authors declare no potential conflict of interest.

396 **Figures**

397

398 **Figure 1.** Receiver operating characteristic (ROC) curve for each commercial and in-
399 house assay on dried blood spot specimens. ROC curves are presented for n=10 SARS-
400 CoV-2 antibody negative DBS specimens and n=10 SARS-CoV-2 antibody positive DBS
401 specimens. One 6 mm (1/4 inch) punch was used for the EUROIMMUN assay and two 6

402 mm (1/4 inch) punches were used for the Platelia and in-house assays. Data from four 6
403 mm (1/4 inch) punches is presented for all other assays. EUROIMMUN = Anti-SARS-
404 CoV-2 ELISA assay (EUROIMMUN, Lübeck, Germany). Platelia = SARS-CoV-2 assay
405 (Bio-Rad, Hercules, California). LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia,
406 Italy). COV2G = SARS-CoV-2 COV2G assay (Siemens, Erlangen, Germany). COV2T =
407 SARS-CoV-2 COV2T assay (Siemens). Elecsys S = Quantitative Anti-SARS-CoV-2 assay
408 (Elecsys spike; Roche, Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys
409 nucleocapsid; Roche). VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics,
410 Raritan, New Jersey). Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada).
411 GSP/DELFI = Anti-SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In-
412 house S (U of T) = In-house spike assay (University of Toronto). In-house RBD (U of T)
413 = In-house RBD assay (University of Toronto). In-house N (U of T) = In-house
414 nucleocapsid assay (University of Toronto). In-house S, mono (U of O) = In-house
415 monoclonal spike assay (University of Ottawa). In-house RBD, mono (U of O) = In-
416 house monoclonal RBD assay (University of Ottawa). In-house N, mono (U of O) = In-
417 house monoclonal nucleocapsid assay (University of Ottawa).

Figure 2. Distribution of values obtained for each commercial and in-house assay on dried blood spot (DBS) specimens. Each panel shows the optical density ratio (OD Ratio), arbitrary units per mL (AU/mL), index, units per mL (U/mL), cut-off index, or signal to cut-off ratio (S/Co) for SARS-CoV-2 antibody negative DBS specimens (n=10) represented in blue and SARS-CoV-2 antibody positive DBS specimens (n=10) represented in orange. One 6 mm (1/4 inch) punch was used for the EUROIMMUN assay and two 6 mm (1/4 inch) punches were used for the Platelia and in-house assays.

Data from four 6 mm (1/4 inch) punches is presented for all other assays.

EUROIMMUN = Anti-SARS-CoV-2 ELISA assay (EUROIMMUN, Lübeck, Germany).

Platelia = SARS-CoV-2 assay (Bio-Rad, Hercules, California). LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia, Italy). COV2G = SARS-CoV-2 COV2G assay (Siemens, Erlangen, Germany). COV2T = SARS-CoV-2 COV2T assay (Siemens). Elecsys S = Quantitative Anti-SARS-CoV-2 assay (Elecsys spike; Roche, Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys nucleocapsid; Roche). VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics, Raritan, New Jersey). Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada). GSP/DELFI A = Anti-SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In-house S (U of T) = In-house spike assay (University of Toronto). In-house RBD (U of T) = In-house RBD assay (University of Toronto). In-house N (U of T) = In-house nucleocapsid assay (University of Toronto). In-house S, mono (U of O) = In-house monoclonal spike assay (University of Ottawa). In-house RBD, mono (U of O) = In-house monoclonal RBD assay (University of Ottawa). In-house N, mono (U of O) = In-house monoclonal nucleocapsid assay (University of Ottawa).

Tables

Table 1. SARS-CoV-2 antibody positive and negative plasma used to contrive dried blood spot specimens.

Sample ID	Description	Sample collection, days post infection	EUROIMMUN Anti-SARS-CoV-2		Bio-Rad Platelia SARS-CoV-2	
			OD ratio	Interpretation ¹	OD ₄₅₀ ratio	Interpretation ²
J12	Healthy donor	COVID-19 era	0.20	Negative	0.08	Negative
D17	Healthy donor	COVID-19 era	0.12	Negative	0.11	Negative
J11	Healthy donor	COVID-19 era	0.16	Negative	0.08	Negative
J1	Healthy donor	COVID-19 era	0.15	Negative	0.08	Negative
D142	Healthy donor	COVID-19 era	0.13	Negative	0.09	Negative
J19	Healthy donor	COVID-19 era	0.49	Negative	0.08	Negative
D9	Healthy donor	COVID-19 era	0.18	Negative	0.09	Negative
J30	Healthy donor	COVID-19 era	0.17	Negative	0.10	Negative
J15	Healthy donor	COVID-19 era	0.18	Negative	0.07	Negative
J7	Healthy donor	COVID-19 era	0.41	Negative	0.10	Negative
20-1887	COVID-19 convalescent donor	44	2.45	Positive	≥4.00	Positive

20-1954	COVID-19 convalescent donor	57	2.32	Positive	3.14	Positive
20-1955	COVID-19 convalescent donor	42	1.36	Positive	2.12	Positive
20-1877	COVID-19 convalescent donor	41	4.10	Positive	≥4.00	Positive
20-1878	COVID-19 convalescent donor	51	4.01	Positive	≥4.00	Positive
20-1882	COVID-19 convalescent donor	66	2.90	Positive	≥4.00	Positive
20-1886	COVID-19 convalescent donor	52	4.62	Positive	≥4.00	Positive
20-1879	COVID-19 convalescent donor	36	11.04	Positive	≥4.00	Positive
20-1885	COVID-19 convalescent donor	38	5.78	Positive	≥4.00	Positive
20-1952	COVID-19 convalescent donor	42	4.39	Positive	≥4.00	Positive

Optical density = OD; Optical density for cut-off control R4 = OD₀R4; Not applicable = N/A

*Results were evaluated by calculating the ratio of the OD of the plasma specimen over the OD of the assay calibrator. Results were interpreted as follows: OD ratio <0.8 reported as positive; OD ratio ≥0.8 to <1.1 reported as borderline; OD ratio ≥1.1 reported as positive.

*Results were evaluated by calculating the ratio of the OD of the plasma specimen over the OD of the kit cut-off control R4. Results were interpreted as follows: OD₀R4 ratio <0.8 reported as negative; OD₀R4 ratio ≥0.8 to <1.0 reported as equivocal; OD₀R4 ratio ≥1.0 reported as positive.

Table 2. Commercial and in-house serological tests assessed for SARS-CoV-2 antibody testing on dried blood spot specimens.

Assay	Manufacturer	Site	Antibody class	Target	Elution volume (μL)	Interpretation		
						Negative	Equivocal	Positive
Anti-SARS-CoV-2 ELISA	EUROIMMUN	J.C. Wilt	IgG	S1	500	OD ratio <0.8	OD ratio ≥0.8 <1.1	OD ratio ≥1.1
LIASON SARS-CoV-2	DiaSorin	Cadham	IgG	S1, S2	200	<15.0 AU/mL	N/A	≥15.0 AU/mL
SARS-CoV-2 COV2G	Siemens	U of T (DID)	IgG	S1	300	Index <1.0	N/A	Index ≥1.0
SARS-CoV-2 COV2T	Siemens	U of T (DID)	Total	S1	300	Index <1.0	N/A	Index ≥1.0
Architect SARS-CoV-2	Abbott	McGill	IgG	N	180	S/Co <1.40	N/A	S/Co ≥1.40
GSP/DELFLIA Anti-SARS-CoV-2	Perkin Elmer	CHEO	IgG	S1	N/A ^a	S/Co <1.4	N/A	S/Co ≥1.4
Platelia SARS-CoV-2	Bio-Rad	J.C. Wilt	Total	N	130	OD ratio <0.8	OD ratio ≥0.8 <1.0	OD ratio ≥1.1
Elecsys Anti-SARS-CoV-2	Roche	NML	Total	S	250	<0.8 U/mL	N/A	≥0.8 U/mL
Elecsys Anti-SARS-CoV-2	Roche	NML	Total	N	250	Cutoff index <1.0	N/A	Cutoff index ≥1.0
VITROS Anti-SARS-CoV-2	Ortho Clinical Diagnostics	BCCDC	Total	S1	300	S/Co <1.0	N/A	S/Co ≥1.0
Abe KT, <i>et al.</i> 2020 ³⁰	In-house	U of T	IgG	S	100	S/Co <1.0	N/A	S/Co ≥1.0
Abe KT, <i>et al.</i> 2020 ³⁰	In-house	U of T	IgG	RBD	100	S/Co <1.0	N/A	S/Co ≥1.0
Abe KT, <i>et al.</i> 2020 ³⁰	In-house	U of T	IgG	N	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (polyclonal)	S	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (monoclonal)	S	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (polyclonal)	RBD	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (monoclonal)	RBD	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (polyclonal)	N	100	S/Co <1.0	N/A	S/Co ≥1.0
Adapted from Amanat F, <i>et al.</i> 2020 ³⁴	In-house	U of O	IgG (monoclonal)	N	100	S/Co <1.0	N/A	S/Co ≥1.0

^aThe elution volume was based on the minimum volume required to perform the assay, the assay's dead volume, and an additional 30 μL of buffer for each 6 mm (1/4 inch) DBS punch to account for liquid absorbed by the filter paper.

^aDBS punches shipped directly to testing site. The GSP/DELFLIA performs automated, onboard elution.

National Microbiology Laboratory at the J.C. Wilt Infectious Diseases Research Centre, Winnipeg, Canada = J.C. Wilt; Division of Infectious Diseases, University of Toronto = U of T (DID); McGill University Health Centre, McGill University = McGill; Children's Hospital of Eastern Ontario, Ottawa, Canada = CHEO; National Microbiology Laboratory at the Canadian Science Centre for Human and Animal Health, Winnipeg, Canada = NML; British Columbia Centre for Disease Control, Vancouver, Canada = BCCDC; University of Toronto, Toronto, Canada = U of T; University of Ottawa, Ottawa, Canada = U of O; Spike S1 = S1; Spike S2 = S2; Total spike = S; Nucleocapsid = N; Receptor binding domain = RBD; Optical density = OD; Signal cut-off = S/Co; Arbitrary units/mL = AU/mL; Not applicable = N/A

Table 3. Assay performance for SARS-CoV-2 antibody testing on dried blood spot specimens.

Assay	DBS punches (n=)	TP (n=)	FN (n=)	TN (n=)	FP (n=)	Eq (n=)	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
EUROIMMUN	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
Platelia	2	10	0	9	0	1	100% (72.2, 100)	90% (59.6, 99.5)	90.9% (62.3, 99.5)	100% (70.1, 100)
LIASON	2	1	9	10	0	0	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	52.6% (31.7, 72.7)
LIASON	3	1	9	10	0	0	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	52.6% (31.7, 72.7)
LIASON	4	1	9	10	0	0	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	52.6% (31.7, 72.7)
COV2G	2	1	9	0	10	1	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	53% (32, 73)
COV2G	3	1	9	0	10	1	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	53% (32, 73)
COV2G	4	1	9	2	8	1	10% (0.5, 40.4)	80% (49, 96.4)	33.3% (1.7, 88.2)	47.1% (26.2, 69)
COV2T	2	1	9	10	0	0	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	52.6% (31.7, 72.7)
COV2T	3	3	7	9	1	0	30% (10.8, 60.3)	90% (59.6, 99.5)	75% (30.1, 98.7)	56.3% (33.2, 76.9)
COV2T	4	4	6	6	4	0	40% (16.8, 68.7)	60% (31.3, 83.2)	50% (21.5, 78.5)	50% (25.4, 74.6)
Elecsys S	2	9	1	0	10	0	90% (59.6, 99.5)	100% (72.2, 100)	100% (70.1, 100)	90.9% (62.3, 99.5)
Elecsys S	3	9	1	0	10	0	90% (59.6, 99.5)	100% (72.2, 100)	100% (70.1, 100)	90.9% (62.3, 99.5)
Elecsys S	4	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)

Elecsys N	2	6	4	10	0	0	60% (31.3, 83.2)	100% (72.2, 100)	100% (72.2, 100)	71.4% (45.4, 88.3)
Elecsys N	3	7	3	10	0	0	70% (39.7, 89.2)	100% (72.2, 100)	100% (39.7, 89.2)	76.9% (49.7, 91.8)
Elecsys N	4	9	1	10	0	0	90% (59.6, 99.5)	100% (72.2, 100)	100% (70.1, 100)	90.9% (62.3, 99.5)
VITROS	2	10	0	0	10	0	100% (72.2, 100)	0% (0, 27.8)	50% (29.9, 70.1)	N/A
VITROS	3	10	0	0	10	0	100% (72.2, 100)	0% (0, 27.8)	50% (29.9, 70.1)	N/A
VITROS	4	10	0	0	10	0	100% (72.2, 100)	0% (0, 27.8)	50% (29.9, 70.1)	N/A
Architect	2	1	9	0	10	0	10% (0.5, 40.4)	100% (72.2, 100)	100% (5.1, 100)	52.6% (31.7, 72.7)
Architect	3	2	8	0	10	0	20% (3.6, 51)	100% (72.2, 100)	100% (17.8, 100)	55.6% (33.7, 75.4)
Architect	4	2	8	0	10	0	20% (3.6, 51)	100% (72.2, 100)	100% (17.8, 100)	55.6% (33.7, 75.4)
GSP/DELFI	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house S (U of T)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house RBD (U of T)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house N (U of T)	2	4	6	10	0	0	40% (16.8, 68.7)	100% (72.2, 100)	100% (51.0, 100)	62.5% (38.6, 81.5)
In-house S, polyclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house RBD, polyclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house N, polyclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house S, monoclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house RBD, monoclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
In-house N, monoclonal (U of O)	2	10	0	10	0	0	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)

University of Toronto = U of T; University of Ottawa = U of O; True positive = TP; False negative = FN; True negative = TN; False positive = FP; Equivocal = Eq; Sensitivity = Se; Specificity = Sp; Confidence

interval = CI; Dried blood spot = DBS.

Table 4. Area under the receiver operating characteristic curve for each commercial and in-house assay on dried blood spot specimens.

Assay	DBS punches (n=)	Area (95% CI)	P value
EUROIMMUN	2	1.0 (1.0, 1.0)	<0.05
Platelia	2	1.0 (1.0, 1.0)	<0.05
LIASON	2	0.60 (0.35, 0.85)	0.45
LIASON	3	0.60 (0.35, 0.85)	0.45
LIASON	4	0.65 (0.40, 0.90)	0.26
COVzG	2	0.59 (0.33, 0.85)	0.50
COVzG	3	0.66 (0.40, 0.91)	0.24
COVzG	4	0.66 (0.39, 0.92)	0.24
COVzT	2	0.59 (0.33, 0.85)	0.50
COVzT	3	0.55 (0.29, 0.81)	0.71
COVzT	4	0.61 (0.35, 0.86)	0.43
Elecsys S	2	0.95 (0.84, 1.0)	<0.05
Elecsys S	3	1.0 (1.0, 1.0)	<0.05
Elecsys S	4	1.0 (1.0, 1.0)	<0.05
Elecsys N	2	1.0 (1.0, 1.0)	<0.05
Elecsys N	3	1.0 (1.0, 1.0)	<0.05
Elecsys N	4	1.0 (1.0, 1.0)	<0.05
VITROS	2	0.93 (0.78, 1.0)	<0.05
VITROS	3	0.93 (0.81, 1.0)	<0.05
VITROS	4	0.96 (0.87, 1.0)	<0.05
Architect	2	1.0 (1.0, 1.0)	<0.05
Architect	3	1.0 (1.0, 1.0)	<0.05
Architect	4	1.0 (1.0, 1.0)	<0.05
GSP/DELFI	2	1.0 (1.0, 1.0)	<0.05
In-house S (U of T)	2	1.0 (1.0, 1.0)	<0.05
In-house RBD (U of T)	2	1.0 (1.0, 1.0)	<0.05
In-house N (U of T)	2	0.98 (0.93, 1.0)	<0.05
In-house S, polyclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05
In-house RBD, polyclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05
In-house N, polyclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05
In-house S, monoclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05
In-house RBD, monoclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05
In-house N, monoclonal (U of O)	2	1.0 (1.0, 1.0)	<0.05

University of Toronto = U of T; University of Ottawa = U of O; Confidence interval = CI; Dried blood spot = DBS; Spike = S; Nucleocapsid = N; Receptor binding domain = RBD.

Additional information

Supplementary Figure 1. Simplified procedure for contriving dried blood spot specimens. This figure was created using BioRender (<https://biorender.com/>).

Supplementary Figure 2. Receiver operating characteristic curve for each commercial and in-house assay on dried blood spot specimens. ROC curves are presented for n=10 SARS-CoV-2 antibody negative DBS specimens and n=10 SARS-

472 CoV-2 antibody positive DBS specimens. One 6 mm (1/4 inch) punch was used for the
473 EUROIMMUN assay and two 6 mm (1/4 inch) punches were used for the Platelia and
474 in-house assays. EUROIMMUN = Anti-SARS-CoV-2 ELISA assay (EUROIMMUN,
475 Lübeck, Germany). Platelia = SARS-CoV-2 assay (Bio-Rad, Hercules, California).
476 LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia, Italy). COV2G = SARS-CoV-2
477 COV2G assay (Siemens, Erlangen, Germany). COV2T = SARS-CoV-2 COV2T assay
478 (Siemens). Elecsys S = Quantitative Anti-SARS-CoV-2 assay (Elecsys spike; Roche,
479 Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys nucleocapsid; Roche).
480 VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics, Raritan, New Jersey).
481 Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada). GSP/DELFIA = Anti-
482 SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In-house S (U of T) = In-
483 house spike assay (University of Toronto). In-house RBD (U of T) = In-house RBD assay
484 (University of Toronto). In-house N (U of T) = In-house nucleocapsid assay (University
485 of Toronto). In-house S, mono (U of O) = In-house monoclonal spike assay (University
486 of Ottawa). In-house RBD, mono (U of O) = In-house monoclonal RBD assay
487 (University of Ottawa). In-house N, mono (U of O) = In-house monoclonal nucleocapsid
488 assay (University of Ottawa).

489 **Supplementary Figure 3.** Distribution of values obtained for each commercial and
490 in-house assay on dried blood spot (DBS) specimens. Distribution of values obtained for
491 each commercial and in-house assay on dried blood spot (DBS) specimens. Each panel
492 shows the optical density ratio (OD Ratio), arbitrary units per mL (AU/mL), index, units
493 per mL (U/mL), cut-off index, or signal to cut-off ratio (S/Co) for SARS-CoV-2 antibody
494 negative DBS specimens (n=10) represented in blue and SARS-CoV-2 antibody positive

495 DBS specimens (n=10) represented in orange. One 6 mm (1/4 inch) punch was used for
 496 the EUROIMMUN assay and two 6 mm (1/4 inch) punches were used for the Platelia
 497 and in-house assays. EUROIMMUN = Anti-SARS-CoV-2 ELISA assay (EUROIMMUN,
 498 Lübeck, Germany). Platelia = SARS-CoV-2 assay (Bio-Rad, Hercules, California).
 499 LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia, Italy). COV2G = SARS-CoV-2
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 502 Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys nucleocapsid; Roche).
 503 VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics, Raritan, New Jersey).
 504 Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada). GSP/DELFIA = Anti-
 505 SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In-house S (U of T) = In-
 506 house spike assay (University of Toronto). In-house RBD (U of T) = In-house RBD assay
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 508 of Toronto). In-house S, mono (U of O) = In-house monoclonal spike assay (University
 509 of Ottawa). In-house RBD, mono (U of O) = In-house monoclonal RBD assay
 510 (University of Ottawa). In-house N, mono (U of O) = In-house monoclonal nucleocapsid
 511 assay (University of Ottawa). In-house S, poly (U of O) = In-house polyclonal spike
 512 assay (University of Ottawa). In-house RBD, poly (U of O) = In-house polyclonal RBD
 513 assay (University of Ottawa). In-house N, poly (U of O) = In-house polyclonal
 514 nucleocapsid assay (University of Ottawa).

515 **Supplementary Table 1.** Raw data generated during this study for each commercial
 516 and in-house assay on dried blood spot specimens.

Figures

Figure 1

Receiver operating characteristic (ROC) curve for each commercial and in house assay on dried blood spot specimens. ROC curves are presented for n=10 SARS CoV-2 antibody negative DBS specimens and n=10 SARS-CoV-2 antibody positive DBS specimens. One 6 mm (1/4 inch) punch was used for the

EUROIMMUN assay and two 6 mm (1/4 inch) punches were used for the Platelia and in-402 house assays. Data from four 6 mm (1/4 inch) punches is presented for all other assays. EUROIMMUN = Anti-SARS CoV-2 ELISA assay (EUROIMMUN, Lübeck, Germany). Platelia = SARS-CoV-2 assay (Bio-Rad, Hercules, California). LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia, Italy). COV2G = SARS-CoV-2 COV2G assay (Siemens, Erlangen, Germany). COV2T = SARS-CoV-2 COV2T assay (Siemens). Elecsys S = Quantitative Anti-SARS-CoV-2 assay (Elecsys spike; Roche, Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys nucleocapsid; Roche). VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics, Raritan, New Jersey). Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada). GSP/DELFI A = Anti-SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In house S (U of T) = In-house spike assay (University of Toronto). In-house RBD (U of T) = In-house RBD assay (University of Toronto). In-house N (U of T) = In-house nucleocapsid assay (University of Toronto). In-house S, mono (U of O) = In-house monoclonal spike assay (University of Ottawa). In-house RBD, mono (U of O) = In house monoclonal RBD assay (University of Ottawa). In-house N, mono (U of O) = In house monoclonal nucleocapsid assay (University of Ottawa).

Figure 2

Distribution of values obtained for each commercial and in-house assay on dried blood spot (DBS) specimens. Each panel shows the optical density ratio (OD Ratio), arbitrary units per mL (AU/mL), index, units per mL (U/mL), cut-off index, or signal to cut-off ratio (S/Co) for SARS-CoV-2 antibody negative DBS specimens (n=10) represented in blue and SARS-CoV-2 antibody positive DBS specimens (n=10) represented in orange. One 6 mm (1/4 inch) punch was used for the EUROIMMUN assay and two 6 mm

(1/4 inch) punches were used for the Platelia and in-house assays. Data from four 6 mm (1/4 inch) punches is presented for all other assays. EUROIMMUN = Anti-SARS-CoV-2 ELISA assay (EUROIMMUN, Lübeck, Germany). Platelia = SARS-CoV-2 assay (Bio-Rad, Hercules, California). LIASON = SARS-CoV-2 assay (DiaSorin, Saluggia, Italy). COV2G = SARS-CoV-2 COV2G assay (Siemens, Erlangen, Germany). COV2T = SARS-CoV-2 COV2T assay (Siemens). Elecsys S = Quantitative Anti-SARS-CoV-2 assay (Elecsys spike; Roche, Basel, Switzerland). Elecsys N = Anti-SARS-CoV-2 assay (Elecsys nucleocapsid; Roche). VITROS = Anti-SARS-CoV-2 assay (Ortho Clinical Diagnostics, Raritan, New Jersey). Architect = SARS-CoV-2 assay (Abbott, Mississauga, Canada). GSP/DELFI A = Anti-SARS-CoV-2 assay (PerkinElmer, Waltham, Massachusetts). In-house S (U of T) = In-house spike assay (University of Toronto). In-house RBD (U of T) = In-house RBD assay (University of Toronto). In-house N (U of T) = In-house nucleocapsid assay (University of Toronto). In-house S, mono (U of O) = In-house monoclonal spike assay (University of Ottawa). In-house RBD, mono (U of O) = In-house monoclonal RBD assay (University of Ottawa). In-house N, mono (U of O) = In-house monoclonal nucleocapsid assay (University of Ottawa).

Supplementary Files

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- [17032021SupplementaryFigures.pdf](#)
- [17032021SupplementaryTable1.docx](#)