Comparative analysis of capillary versus venous blood for serologic detection of SARS-CoV-2 antibodies by rPOC lateral flow tests

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Abstract: A comparison of rapid point of care serology tests using finger prick and venous blood was done on 278 participants. In a laboratory setting, IgG sensitivity neared 100%; however, IgG sensitivity dramatically dropped (82%) in field testing. Possible factors include finger prick volume variability, hemolysis, cassette readability and operator training.

Keywords: COVID-19, Rapid Point of Care Test, Serology, Diagnostic Microbiology, Public Health
As the number of COVID-19 cases continues to increase worldwide, the need for fast, easy-to-use and accurate tests is urgent. Rapid point of care (rPOC) lateral flow tests measure serum antibodies to SARS-CoV-2 and a number of them are currently available. Most cassettes are designed to detect separately and simultaneously IgM and IgG antibody types and they have been used in a variety of studies to provide estimates of population seroprevalence. In acute cases, when a patient is repeatedly negative for PCR but symptomatic, a highly sensitive and specific rPOC could be utilized as a diagnostic method for those with difficult venous access. rPOC can be also be used for surveillance purposes in hard to reach populations that have no access to laboratories, or to satisfy pre-travel requirements. The literature suggests that most of the comparative evaluations on rPOC tests were done in laboratory settings [1-3]. In order to evaluate the performance of rPOC in the field, the British Columbia Centre for Disease Control Public Health Laboratory (BCCDC PHL) conducted a comparative assessment of the performance of rPOC lateral flow assays in a laboratory (using venous blood samples) versus a field (using fingerpick capillary blood) setting. Field testing was conducted in 2 long-term care facilities (LTCFs) affected by COVID-19 outbreaks [4].

We conducted initial laboratory-based evaluations with a total of 142 venous blood samples, with subsequent evaluation in the field on 278 capillary blood samples. Briefly, 3 rPOC products were screened in the laboratory using venous samples obtained from known COVID-19 patients at 0-7, 8-14 and >14 days post-illness onset (total n=79), as well as pre-pandemic negative samples stored at BCCDC PHL tested for other serology prior to 2019, which included samples with seropositivity to other common pathogens, such as HIV, HCV, syphilis, etc. (n=63) (Table 1). Some of those negative samples, such as Toxoplasma IgM, West Nile Virus IgM, Chikungunya IgM were selected because they are notorious for exhibiting non-specific reactivity against many other pathogens (Table 2). All rPOC products used in this study could detect both IgM and IgG on the same cassette. Positive patients were confirmed by BCCDC PHL in-house laboratory developed RT PCR [5]. Three of the products tested – Artron Diagnostics Inc. (Canada) referred as Artron, BioCan Diagnostics Inc. (Canada) referred as BioCan and Rapid Response BTNX (China) referred as BTNX – yielded very promising analytical performance with 91%-95% sensitivity and 93%-100% specificity (Table 1). All
3 assays demonstrated highest sensitivities when tested against serum taken >14 days post-illness onset. In terms of specificity, Artron detection of COVID-19 IgM cross-reacted with WNV IgG+, mumps IgM + and Chikungunya IgM + sera and BTNX detection of COVID-19 IgG cross-reacted with Toxoplasma IgM+ serum.

Based on laboratory performance, secure supply chain and product cost, the Artron Diagnostics Inc. product was selected for a dual laboratory/field trial, with the field trial conducted in 2 LTCFs with confirmed COVID-19 outbreaks. In LTCF rPOC tests were performed by trained laboratory medicine technologists, who were trained in their performance prior to conducting testing. The BioCan Diagnostics Inc. and BTNX products were also tested using only the venous blood samples collected at the LTCF.

Samples were collected on from residents and staff at least 14 days after symptoms onset (for known PCR-confirmed COVID-19 patients). Samples comprised those from known COVID-positive patients (PCR-confirmed) and from patients of “unknown” status (either PCR-negative or never tested by PCR). “Unknown” status patients were classified as “presumed positive” (consensus positive SARS-CoV-2 serology on all 4 high throughput automated platforms [1) LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin IgG; DiaSorin, Italy); 2) ARCHITECT SARS-CoV-2 IgG (Abbott IgG; Abbott, USA); 3) VITROS® Anti-SARS-CoV-2 Total (Ortho T) and 4) SARS-CoV-2 Total Assay (Siemens T; Siemens, USA)] and “presumed negative” (consensus negative SARS-CoV-2 serology on all 4 high throughput automated platforms). Any samples with discrepant results on any of the 4 high throughput automated platforms were excluded from the analysis.

In the field, we found that finger-prick-based sensitivity of the Artron rPOC test was overall inferior to that done with venous blood in a laboratory setting (Table 3). Finger prick IgG sensitivity dropped to ~83% (specificity 99%). When paired venous samples were tested in the laboratory on Artron cassettes, the sensitivity did improve to ~89% , but still did not reach that observed in the initial validation study (Table 1). IgM sensitivity in the field setting was higher than in the in-laboratory serum performance on paired samples (~67% vs. ~58%), but both were markedly lower than in the
initial validation study (Table 1). There was also a small drop in specificity for IgM in the field vs.
laboratory setting on paired samples (91.5% vs. 92.6%). When BioCan and BTNX rPOC cassettes
were trialed on a large subset (n dependent on availability of cassettes) of the same venous samples,
the performance was also inferior to that previously observed in the laboratory evaluation.

Specifically, the sensitivity of both BioCan and BTNX cassettes in detecting COVID-19 IgM 14 days
after symptoms-onset was markedly lower than that in the validation study (data not shown).

Furthermore, additional BioCan and BTNX cassettes procured for the LTCF evaluation were noted to
have variable appearance and inferior quality to that of the first batch trialed in the laboratory.

Our results demonstrate poorer performance of rPOC assays under field settings relative to what can
be achieved in the laboratory, possibly due to reduced standardization in blood inoculum in the field.
Capillary blood inoculum may vary in volume with possible effects on sensitivity. The nature of
capillary blood collection also predisposes the sample to hemolysis which might interfere with test
specificity. Variability of lighting in the field and operator training may further compound these
effects. Our experience further highlights the instability of rapidly developed and produced COVID-
19-related product supplies, which can have substantial batch-to-batch variations, depending on the
manufacturer.
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Conflict of Interest: No conflict of interest

Patient Consent Statement:
The study was authorized by the Provincial Health Officer and approved by the Clinical Research Ethics Board of the University of British Columbia (H20-01089).

Long-term care residents capable of providing informed consent were asked before Health Authority staff attended the site to collect specimens if they agreed to participate in the validation work. For residents incapable of providing their own informed consent (the majority of residents), long-term care facility staff contacted the appropriate family members to obtain verbal consent. Long-term care facility staff obtained consent because of their pre-existing relationship with residents and their family members.

Health Authority staff (medical laboratory assistants for phlebotomy and medical laboratory technologists for point-of-care testing) were provided with the list of consented residents. At the time of specimen collection, the resident was asked if it was okay to collect the specimens. No samples were collected or testing performed for any resident who refused at this point, regardless of previous consent from the resident or family members.
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Table 1: Laboratory validation of rPOC COVID-19 lateral flow cassette performance

| Isotype | Test Name | Sensitivity (≤4 to 7) | Sensitivity (>7 to 14) | Sensitivity (>14) | Sensitivity (N=79**) | Specificity (N=63) |
|---------|-----------|-----------------------|------------------------|------------------|----------------------|------------------|
| IgM     | Artron    | 72.7                  | 96.4                   | 100              | 94.9                 | 93.5             |
| IgG     | Artron    | 72.7                  | 89.3                   | 100              | 92.4                 | 100              |
| IgM     | BioCan    | 72.7                  | 89.3                   | 96.9             | 91.1                 | 100              |
| IgG     | BioCan    | 72.7                  | 92.9                   | 100              | 93.7                 | 100              |
| IgM     | BTNX      | 72.7                  | 96.4                   | 93.8             | 91.1                 | 100              |
| IgG     | BTNX      | 63.6                  | 92.9                   | 100              | 92.4                 | 98.4             |

* Total N is not equal to the sum of individual subsets as additional samples with unknown date of onset from illness are included.
Table 2: Specificity assessment of COVID-19 Point of Care test kits

| Negative Samples* | Samples | Arton | Bio-Can | BTNX |
|-------------------|---------|-------|---------|------|
|                   |         | IgM   | IgG     | IgM  | IgG  |
| Presumed Negative** | 19      | 0     | 0       | 0    | 0    | 0    | 0    |
| Coronavirus Seasonal | 2       | 0     | 0       | 0    | 0    | 0    | 0    |
| Coronavirus 229E   | 1       | 0     | 0       | 0    | 0    | 0    | 0    |
| Coronavirus NL63   | 2       | 0     | 0       | 0    | 0    | 0    | 0    |
| Coronavirus HKU1   | 1       | 0     | 0       | 0    | 0    | 0    | 0    |
| SARS-CoV-1         | 2       | 0     | 0       | 0    | 0    | 0    | 0    |
| Influenza A        | 3       | 0     | 0       | 0    | 0    | 0    | 0    |
| Influenza B        | 3       | 0     | 0       | 0    | 0    | 0    | 0    |
| RSV               | 2       | 0     | 0       | 0    | 0    | 0    | 0    |
| HCV +             | 5       | 0     | 0       | 0    | 0    | 0    | 0    |
| Mumps IgM +       | 5       | 1     | 0       | 0    | 0    | 0    | 0    |
| WNV IgM +         | 2       | 1     | 0       | 0    | 0    | 0    | 0    |
| Toxoplasma IgM +  | 5       | 0     | 0       | 0    | 0    | 0    | 0    | 0    | 1    |
| Chikungunya IgM + | 5       | 1     | 0       | 0    | 0    | 0    | 0    |
| RPR 1:512 (Syphilis) | 2  | 0     | 0       | 0    | 0    | 0    | 0    |
| RPR 1:128 (Syphilis) | 2  | 0     | 0       | 0    | 0    | 0    | 0    |
| RPR 1:32 (Syphilis) | 2  | 0     | 0       | 0    | 0    | 0    | 0    |
| Total             | 63      | 3     | 0       | 0    | 0    | 0    | 1    |

*Samples were selected to include those with possible non-specific cross-reactivity; all samples were collected pre-November 2019, prior to SARS-CoV-2 virus being detected in British Columbia.
** Presumed Negatives were selected from samples of prenatal and organ donor.
Table 3: Field trial of rPOC COVID-19 lateral flow cassette performance

|                      | Field Finger prick (capillary sample) | Laboratory Serum (venous sample) |
|----------------------|---------------------------------------|----------------------------------|
|                      | IgM                                   | IgG                              | IgM                              | IgG                              |
|                      | Reactivity                             | Performance                       | Reactivity                       | Performance                       | Reactivity                       | Performance                       | Reactivity                       | Performance                       |
| Artron               |                                       |                                  |                                  |                                  |                                  |                                  |                                  |                                  |
| Known Positive       | 50 / 79                                | 66.7%                            | 65 / 79                          | 82.9%                            | 41/79                            | 58.1%                            | 69/79                            | 88.6%                            |
| Presumed Positive    | 20 / 26                                | 91.5%                            | 22 / 26                          |                                 | 20/26                            | 92.6%                            | 24/26                            | 99.4%                            |
| Presumed Negative    | 15 / 177                               | 91.5%                            | 1 / 177                          |                                 | 13/177                           | 92.6%                            | 1/177                            | 99.4%                            |
| BioCan               |                                       |                                  |                                  |                                  |                                  |                                  |                                  |                                  |
| Known Positive       | Not assessed                           |                                  | 64/78                            | 83.6%                            | 73/78                            | 83.6%                            | 74/78                            | 92.5%                            |
| Presumed Positive    |                                      |                                  | 23/26                            | 86.5%                            | 25/26                            | 86.5%                            | 97.5%                            |                                  |
| Presumed Negative    | 16/119                                 | 86.5%                            | 3/119                            | 97.5%                            |                                  |                                  |                                  |                                  |
| BTNX                 |                                       |                                  |                                  |                                  |                                  |                                  |                                  |                                  |
| Known Positive       | Not assessed                           |                                  | 60/80                            | 72.9%                            | 74/80                            | 72.9%                            | 92.5%                            |                                  |
| Presumed Positive    |                                      |                                  | 18/27                            | 98.8%                            | 25/27                            | 98.8%                            | 99.4%                            |                                  |
| Presumed Negative    | 2/169                                  | 98.8%                            | 1/169                            | 99.4%                            |                                  |                                  |                                  |                                  |