Performance of the T-SPOT®.*COVID* test for detecting SARS-CoV-2-responsive T cells

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A B S T R A C T

Objective: To evaluate the performance of the T-SPOT.*COVID* test for identifying SARS-CoV-2-responsive T-cells in participants with SARS-CoV-2 infection.
Methods: The T-SPOT.*COVID* test uses ELISpot interferon-gamma release assay (IGRA) methodology to measure T cell responses to SARS-CoV-2 spike S1 and nucleocapsid peptides. T-SPOT.*COVID* and anti-N immunoglobulin (Ig) G serology tests were performed on blood from 186 patients with nucleic acid amplification test (NAAT)-confirmed-SARS-CoV-2 infection and 100 control group participants.
Results: In the 2–8 weeks after NAAT-diagnosed SARS-CoV-2 infection, the T-SPOT.*COVID* test detected 98.4% (63 of 64) of infected participants, while anti-N IgG serology detected 82.8%. In the first 2 weeks after diagnosis, during adaptive immune response activation, there were less reactive T-SPOT.*COVID* responses (75.7%, 28 of 37 infected participants) and many less seropositive responses (32.4%). Response numbers tapered after 8 weeks; however, T-SPOT.*COVID* test continued to detect most participants with confirmed infection (83.6%, 56 of 67) and continued to out-perform serology (52.2%). T-SPOT.*COVID* response due to cross-reactive T cells was ruled out by demonstrating that, of 44 control group participants with T cells responsive to 4 human common cold coronavirus peptides, only 1 was T-SPOT.*COVID* reactive.
Conclusion: The T-SPOT.*COVID* test performed well in detecting SARS-CoV-2-sensitized T-cells over many months.

Introduction

Long-term protection from infectious agents, such as the SARS-CoV-2 virus, is mediated by T cells and antibody-mediated immunity of the adaptive immune system (Sette and Crotty, 2021). The T-SPOT.*COVID* test was developed to identify the presence of SARS-CoV-2-responsive T cells.

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T cells contribute to the understanding of SARS-CoV-2 infections in many ways. T cells can identify past SARS-CoV-2 infections at a time when PCR tests would be negative and antibodies levels may be waning (Dan et al., 2021; Gudbjartsson et al., 2020; Poland et al., 2020). T cells can provide immune memory lasting for months (Dan et al., 2021) and perhaps years, as suggested by the discovery of T cells to the SARS-CoV-1 coronavirus 17 years after infection (Le Bert et al., 2020). T cells may act independently of antibodies to control a SARS-CoV-2 infection, as shown by the recovery of COVID-19 patients who lack detectable antibodies but have SARS-CoV-2-responsive T cells (Gallais et al., 2021; Sekine et al., 2020). T cells also show reactivity to numerous SARS-CoV-2 epitopes, so have the potential to protect against many SARS-CoV-2 variants (Grifoni et al., 2020; Tarke et al., 2021). T cell-based assays can probe the longevity of an immune response following a SARS-CoV-2 infection or vaccination (Goletti et al., 2021; Liu et al., 2021; Reynolds et al., 2021). These various roles suggest

that a T cell assay can be a key contributor to SARS-CoV-2 investigations.

The T-SPOT.*COVID* test, an enzyme-linked immunospot (ELISpot) assay, identifies T cells in peripheral blood that release interferon-gamma (IFN-γ) in response to stimulation with SARS-CoV-2 peptides. The T-SPOT.*COVID* test builds on the T-SPOT platform (Oxford Immunotec) used worldwide for tuberculosis and cytomegalovirus testing and the research version, the T-SPOT *Discovery* SARS-CoV-2 test (Liu et al., 2021; Wyllie et al., 2021). The T-SPOT.*COVID* ELISpot methodology is performed in many laboratories and offers a standardized comparison of T cell immunity among participants. In addition, ELISpot assays normalize the number of peripheral blood mononuclear cells (PBMCs), thus maintaining test effectiveness in participants with lymphopenia, a commonly reported condition in many COVID-19 patients (Altmann and Boyton, 2020) and immunosuppressed people.

The objective of this study was to evaluate the ability of the T-SPOT.*COVID* test to detect T cell responses in participants with or without a history of SARS-CoV-2 infection and to compare the T-SPOT.*COVID* test results with anti-N immunoglobulin (Ig)G serology results in the first several months after infection.

Materials and Methods

2.1. Participant recruitment

Participants for this single-center, cross-sectional study were recruited from patients who had attended the outpatient Primacare medical center in Fall River, Massachusetts, USA, between November 30, 2020, and March 24, 2021, a time of high demand for COVID-19 testing. Among other healthcare services, Primacare provided COVID-19 testing to anyone wanting or required to be tested. The New England Center for Clinical Research (NECCR) invited participants to join the study if they had received a positive SARS-CoV-2 nucleic acid amplification test (NAAT) at Primacare or if NECCR deemed them to be at low risk of SARS-CoV-2 infection. As this study was run independently from the participants' healthcare providers, clinical data such as chest x-rays and hospitalizations records were not obtained. Informed consent and study approval were obtained from the Advarra institutional review board by NECCR at Primacare.

Confirmed-infection group: A NAAT, which detects the presence of the SARS-CoV-2 virus, was used to identify people infected with SARS-CoV-2 at the time of testing (Rai et al., 2021). Participants in the confirmed-infection group were recruited from asymptomatic and symptomatic patients who had had a positive SARS-CoV-2 NAAT result within the past 9 months. The date of the first positive NAAT result was considered the date of diagnosis of SARS-CoV-2 infection. Blood was drawn for Abbott SARS-CoV-2 chemiluminescent microparticle immunoassay (CMIA) anti-N IgG serology and T-SPOT.*COVID* tests between 0 to 249 days after diagnosis.

The analysis of responses was divided into 3 time periods: 0 to 2 weeks after diagnosis (0 to 14 days); 2+ to 8 weeks after diagnosis (15 to 56 days); and 8+ weeks after diagnosis (57+ days).

Control group: Many SARS-CoV-2 studies use frozen pre-pandemic blood for control samples; however, the T-SPOT platform requires fresh blood to ensure consistent results. Therefore fresh blood was obtained from control group participants prospectively recruited from individuals with low risk of prior SARS-CoV-2 infection. Requirements for enrollment included no current or prior signs or symptoms of COVID-19, no known contact with a confirmed SARS-CoV-2-infected individual, no prior history of a positive SARS-CoV-2 NAAT, no SARS-CoV-2 vaccination, and no prior diagnosis with SARS-CoV-1 or Middle Eastern Respiratory Syndrome (MERS). In addition, the BIOHIT HealthCare SARS-CoV-2 lateral flow anti-N IgM/IgG serology test was performed at en-

rollment, and the 1 person with a positive BIOHIT result was not enrolled. Blood was drawn at enrollment for testing with T-SPOT.*COVID* and the Abbott CMIA anti-N IgG serology test and anyone with a positive serology result was excluded from the control group.

2.2. T-SPOT.COVID test

The T-SPOT.*COVID* test includes over 250 SARS-CoV-2 peptides (15-mer peptides overlapping by 11 amino acids) in 2 antigen peptide pools; one pool contains peptides from the spike S1 protein, including the receptor-binding domain, and the other contains peptides from the nucleocapsid protein.

Blood samples for the T-SPOT.*COVID* test were processed and analyzed according to the manufacturer's instructions. Briefly, blood samples were drawn into lithium heparin tubes which were shipped overnight to Oxford Immunotec (Abingdon, UK) in temperature-controlled shipping boxes. Next, the T-Cell *Xtend* reagent (Oxford Immunotec) was added to the samples, and PBMCs were isolated by density gradient centrifugation, washed, counted, and 250 000 cells/well were plated into 4 wells of a 96-well plate.

The 2 antigen peptide pools were added to the 2 antigen wells; the T cell mitogen phytohemagglutinin to the positive control well, and cell culture media alone to the negative control well. After 16–20 hours incubation, the wells were washed and developed using a conjugated secondary antibody that bound to any IFN-γ captured on the membrane. After washing to remove unbound IFN-γ, substrate was added to produce dark spots of insoluble precipitate indicating areas of IFN-γ secretion from T cells. These spot forming cells (SFCs) were counted using an automated ELISpot plate reader (CTL, Shaker Heights, OH) and manually verified. Results were 'invalid' if the negative control had more than 10 SFCs or the positive control had fewer than 20 SFCs when the antigen wells were non-reactive. The test cut-off was predetermined at 6 SFCs using a receiver operating characteristic (ROC) curve derived from confirmed-infection and low-risk samples from another cohort (data not shown), as that cut-off gave the optimal sensitivity and specificity for the test. A borderline zone of +/-1 SFCs was introduced to account for potentially elevated test variability around the cut-off (Rego et al., 2018). Consequently, results were 'reactive' when the SFCs in the higher of the 2 antigen wells minus the negative control were ≥ 8, 'non-reactive' when the SFCs in both antigen wells minus the negative control were ≤ 4, and 'borderline' when the SFCs in the higher of the antigen wells minus the negative control were 5, 6 or 7. The SFC results presented below refer to counts from the higher of the 2 antigen wells.

2.3. Cross-reactivity analysis

Due to homology between virus sequences, common cold human coronavirus (HCoV) endemic strains can generate T cells capable of cross-reacting with some SARS-CoV-2 peptides (Braun et al., 2020; Mateus et al., 2020). To minimize the chance of T-SPOT.*COVID* responses being elicited by cross-reactive T cells in the absence of a SARS-CoV-2 infection, the T-SPOT.*COVID* test omits peptides from the S2 region of the SARS-CoV-2 spike protein, as the majority of SARS-CoV-2 / HCoV homologous sequences are in this region (Braun et al., 2020). In addition, a sequencing search tool and genetic sequence database were used to identify sequences in the spike, nucleocapsid, membrane, and envelope proteins with high amino acid homology between SARS-CoV-2 and the HCoVs; any spike S1 and nucleocapsid high homology sequences identified in this way were also omitted from the T-SPOT.*COVID* test.

To explore potential cross-reactive T cell responses, 5 'cross-reactivity' peptide pools were manufactured, consisting of a 'high

Table 1
Participant characteristics [a]

	Confirmed-infection group (n=186)	Control group (n=100)
Gender		
Female, n (%)	114 (61.3%)	64 (64%)
Male, n (%)	72 (38.7%)	36 (34%)
Age, years		
Median (Min, Max)	52 (19-83)	56 (18-87)
Country of Birth		
USA	128	83
Portugal	48	10
Puerto Rico	3	3
Cape Verde	2	1
Brazil	1	0
Ecuador	1	0
Germany	1	0
Korea	1	1
Lebanon	1	1
Mexico	0	1
COVID-19 symptoms, n (%)		
New, continuous cough	110 (59.1%)	N/A
Fever	90 (48.4%)	N/A
Shortness of breath	82 (44.1%)	N/A
Sore throat	70 (37.6%)	N/A
Runny nose	88 (47.3%)	N/A
Headache	119 (64.0%)	N/A
Muscle aches	108 (58.1%)	N/A
Altered sense of smell	104 (55.9%)	N/A
Altered sense of taste	109 (58.6%)	N/A
Extreme fatigue	120 (64.5%)	N/A
Diarrhea	62 (33.3%)	N/A
Vomiting	17 (9.1%)	N/A
Itchy red patches on digits	1 (0.5%)	N/A

[a] For participants with reactive, non-reactive, and borderline results

homology' pool containing the identified highly homologous peptides and 4 'HCoV pools' containing spike peptides from 4 HCoVs (HKU1, 229E, NL63 and OC43). For the cross-reactivity analysis, after subtracting the negative control SFCs, the T-SPOT.*COVID* cut-off of ≥6 SFCs defined a reactive response and <5 SFCs defined a non-reactive response.

2.4. Statistical analysis

Receiver operating characteristic (ROC) curve analysis by Graph-Pad Prism 9.1.1 for Windows was used to evaluate the SFC separation between reactive and non-reactive T-SPOT.*COVID* results. Other statistical analyses were performed using SAS version 9.4, SAS Institute, Cary NC, USA. The maximum SFC counts for the control and confirmed-infection groups were compared using Pearson's test with Yate's correction. Control group participant responses to each of the 5 cross-reactivity peptide pools were compared with their T-SPOT.*COVID* response using the McNemar test. A *P*-value of <0.05 was considered significant.

Results

3.1. Participant groups

Of 204 participants initially screened for the confirmed-infection group, 17 were excluded because results for the anti-N IgG serology test were not available and 1 was excluded due to an insufficient number of PBMCs to perform the T-SPOT.*COVID* test. The remaining 186 confirmed-infection group participants, 114 women and 72 men, ranged from 19 to 83 years old (Table 1). The majority of these participants (176 of 186, 94.6%) had symptomatic COVID-19 (Table 1), with the remainder having NAAT-confirmed asymptomatic SARS-CoV-2 infections.
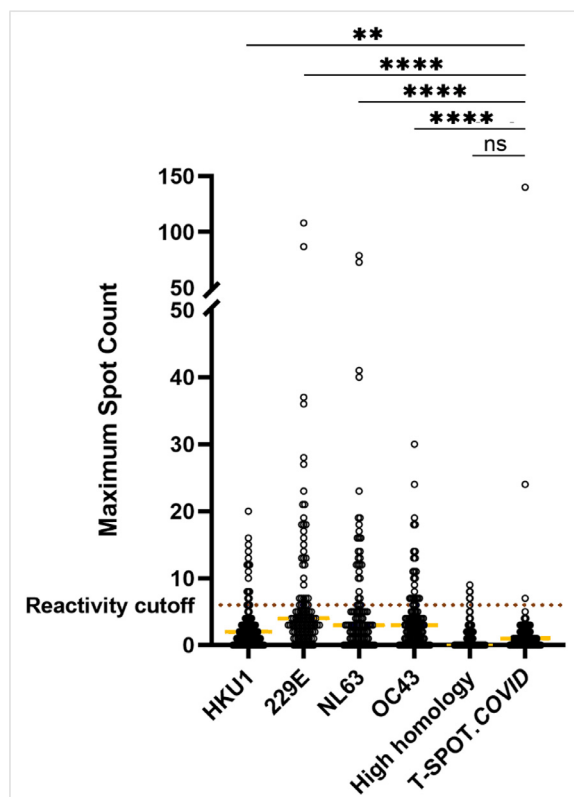

Figure 1. T cell response to HCoVs and T-SPOT.*COVID* peptides.
Response of 100 control group participants to the spike peptides of 4 human common cold coronaviruses (HCoVs), HKU1, 229E, NL63, OC43, and to high homology peptides from structural proteins of these HCoVs. Median response shown with solid yellow line. Reactivity cut-off shown with dotted brown line. Participants with borderline T-SPOT.*COVID* results included. Spot forming cell (SFC) count is the number of SFCs per well (# per 2.5×10^5 peripheral blood mononuclear cells) minus the negative control. Y-axis scale changes at 50. **$P=0.0011$, ****$P<0.0001$, ns: not significant.

Of the 112 participants initially screened for the control group, 12 were excluded: 6 due to positive anti-N IgG serology results, 4 due to unavailable anti-N serology results, 1 due to insufficient number of PBMCs, and 1 due to technical error in test performance. The remaining 100 control group participants, 64 women and 36 men, ranged from 18 to 87 years old (Table 1).

3.2. Cross-reactivity analysis

The peptides included in the T-SPOT.*COVID* test were selected to minimize the chance for cross-reactivity from T cells sensitized by a prior HCoV infection. The success of this strategy was evaluated by testing the control group participant responses to the 5 cross-reactivity pools (4 HCoV peptide pools and the high homology peptide pool).

The majority of control group participants (56 of 100, 56.0%) did not respond to any of the 5 cross-reactivity pools, while 44 of 100 (44%) had responses (Figure 1). Not all of these 44 control group participants responded to each cross-reactivity pool: the HKU1 pool elicited responses from 18%, 229E from 34%, NL63 from 29%, OC43 from 26%, and the high homology pool from 6%.

Of the 44 control group participants who responded to the cross-reactivity peptides, only 1 responded to the T-SPOT.*COVID* peptides, while 98% (43 of 44) of participants with proven HCoV-responsive T cells did not show T-SPOT.*COVID* reactivity.

The above results show that HCoV-responsive T cells did not lead to reactive T-SPOT.*COVID* results. The converse, that re-

active T-SPOT.*COVID* results did not necessarily signify HCoV-responsiveness, was also observed: of the 3 control group participants with T-SPOT.*COVID* results ≥6 SFCs (1 borderline, 2 reactive), 2 were HCoV-non-responsive and 1 was HCoV-responsive.

3.3. T-SPOT.COVID *ROC curve, invalid, and borderline results*

A ROC curve analysis was conducted using the 186 confirmed-infection and 100 control group participants which confirmed the appropriateness of the pre-determined 6 SFC cut-off and yielded an area under the curve (AUC) of 0.95 (95% CI: 0.92 to 0.98) (Figure 2A).

No invalid T-SPOT.*COVID* results occurred in either the confirmed-infection or control group.

Borderline results were seen in 18 (9.7%) of the 186 confirmed-infection and 2 (2%) of the 100 control group participants. The timing of the test may have influenced the borderline results in the confirmed-infection group (see Discussion). As borderline results are neither reactive nor non-reactive, participants with borderline results were not included in the following analysis which focused on reactive and non-reactive T-SPOT.*COVID* results.

3.4. T-SPOT.COVID *reactive and non-reactive results*

A total of 98 control group participants had T-SPOT.*COVID* reactive and non-reactive results; of these, 98.0% (96 of 98) had non-reactive T-SPOT.*COVID* results and, as predetermined by the inclusion criteria, 100% were anti-N IgG seronegative.

A total of 168 confirmed-infection group participants had T-SPOT.*COVID* reactive and non-reactive results. In the 2+ to 8 weeks after diagnosis, 98.4% (63 of 64) of confirmed-infection group participants had reactive T-SPOT.*COVID* results and 82.8% (53 of 64) were anti-N IgG seropositive.

The SFC count ranged from 0 to 234 (median 29) in the confirmed-infection group participants and from 0 to 4 (median 1) in the 96 control group participants with non-reactive results (Figure 2B). In the 2 control group participants with reactive T-SPOT.*COVID* results, 1 had 24 SFCs and the other 140 SFCs; counts near or well above the median for the confirmed-infection group participants.

### 3.5. T-SPOT.COVID *and serology results according to time after diagnosis*

Within the first 2 weeks after diagnosis, 75.7% (28 of 37) of confirmed-infection group participants had reactive T-SPOT.*COVID* results and 32.4% (12 of 37) had positive anti-N IgG serology results (Figure 3A). Between 2+ to 8 weeks after diagnosis, 98.4% (63 of 64) had reactive T-SPOT.*COVID* results and 82.8% (53 of 64) had positive serology results. More than 8 weeks after diagnosis, 83.6% (56 of 67) had reactive T-SPOT.*COVID* results and 52.2% (35 of 67) had positive serology results. Subdividing this latter period into 3 periods of 8+ to 14 weeks, 14+ to 20 weeks, and 20+ to 36 weeks, T-SPOT.*COVID* reactivity was 86.1%, 91.7%, and 73.7%, respectively, and anti-N IgG serology was 86.1%, 33.3%, and 0%, respectively (Figure 3A).

Reactive T-SPOT.*COVID* results were observed in 6 of the 9 asymptomatic participants: 1 of 3 tested in the first 2 weeks, 4 of 4 between 2+ to 8 weeks, and 1 of 2 after 8 weeks. The serology tests were positive in 4 of the 9 asymptomatic participants: 1 of 3 tested in the first 2 weeks, 3 of 4 between 2+ to 8 weeks, and 0 of 2 after 8 weeks.

3.6. Overlap of T-SPOT.COVID *and serology responses*

Among the 168 confirmed-infection group participants, 152 (90.5%) were detected by the T-SPOT.*COVID* test and/or the serology
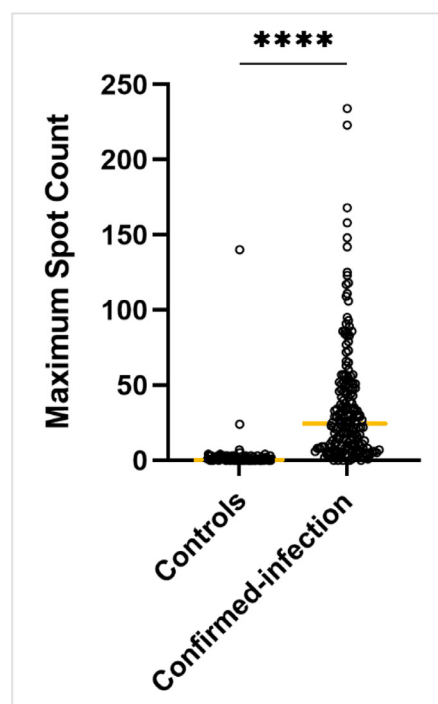
A
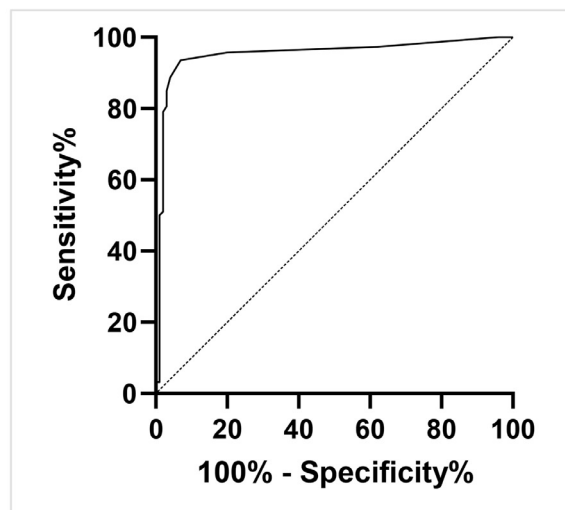
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Figure 2. A. Receiver operating curve (ROC) for T-SPOT.*COVID* participants: ROC curve for T-SPOT.*COVID* results from confirmed-infection (n=186) and control (n=100) group participants. Area under curve = 0.95 (95% CI: 0.92 to 0.98). Sensitivity % (y-axis) refers to the percent of confirmed-infection group participants who are also T-SPOT.*COVID* reactive. Specificity% (x-axis) refers to the percent of low-SARS-CoV-2-risk control group participants who are also T-SPOT.*COVID* non-reactive. **B. T-SPOT.*COVID* maximum spot counts:** Maximum number of spot forming cells (SFCs) (maximum of the 2 T-SPOT.*COVID* antigen wells) for confirmed-infection (n=186) and control group participants (n=100). SFC count is the number of SFCs per well (# per 2.5×10^5 peripheral blood mononuclear cells) minus the negative control. Median response shown with solid yellow line. ****$P<0.0001$.
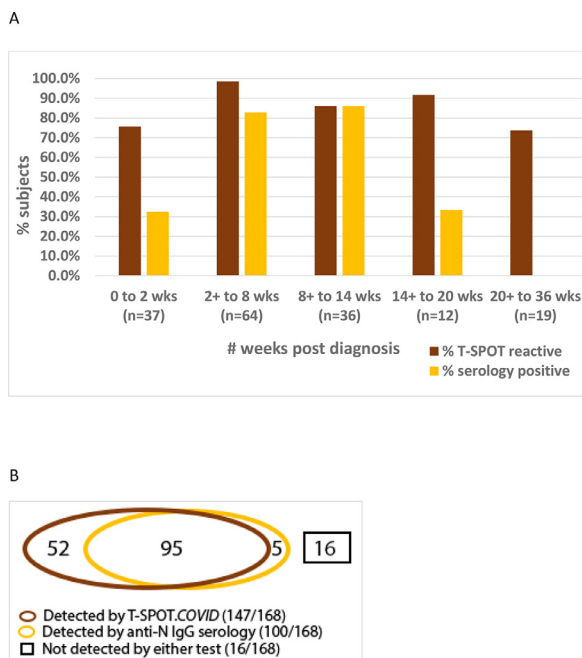
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Figure 3. (A) **Comparison of T-SPOT.***COVID* **and serology results by time period:** Percentage of reactive T-SPOT.*COVID* results and positive serology results in confirmed-infection group participants (n=168) according to number of weeks after SARS-CoV-2 NAAT positive result. Participants with borderline T-SPOT.*COVID* results are not included. Note: No participants were recruited between 12 and 15 weeks post diagnosis due to the timing of participant recruitment and lulls in regional COVID-19 cases and polymerase chain reaction testing. (B) **Number of participants detected by T-SPOT.***COVID* **and serology tests:** Venn diagram showing that the combined results of the T-SPOT.*COVID* and anti-N serology tests detect more confirmed-infection group participants than either test alone. The number of participants detected by the T-SPOT.*COVID* test shown in the brown circle, the number detected by the anti-N IgG serology test shown by the yellow circle, and the number not detected by either test shown in the black rectangle. Participants with borderline T-SPOT.*COVID* results are not included.

test across all time points. Overall, 95 confirmed-infection group participants were detected by both tests, while the T-SPOT.*COVID* test detected an additional 52 participants not detected by serology, and serology detected 5 participants not detected by the T-SPOT.*COVID* test (Figure 3B). The 16 confirmed-infection group participants not detected by either test were tested during the first 2 weeks following diagnosis (n=9) or more than 8 weeks after diagnosis (n=7).

Discussion

The T-SPOT.*COVID* test was highly accurate at differentiating confirmed-infection and control group participants, as shown by the AUC of 0.95.

Responses to the cross-reactivity peptide pools were observed in 44% of the control group participants, agreeing with reports that 35%–50% of control blood obtained during the SARS-CoV-2 pandemic had cross-reactive T cells (Braun et al., 2020; Le Bert et al., 2020). That 43 of 44 control group participants had HCoV-responsive T cells but non-reactive T-SPOT.*COVID* results demonstrates that the T-SPOT.*COVID* test did not induce cross-reactive responses from these participants' T cells. These findings confirm that reactive T-SPOT.*COVID* results observed in confirmed-infection group participants were generated by T cells activated by the SARS-CoV-2 virus.

The low number of control group participants (n=6) responding to the high homology pool is of interest when compared with the higher number (n=44) responding to the HCoV pools. The high homology sequences, by definition, were contained in the HCoVs that

infected these 44 subjects, so that 86.4% (38/44) of these participants did not show immune responses to the high homology peptides indicates that these peptides were not highly immunogenic. This finding is understandable from an evolutionary viewpoint: An immune response to a viral peptide creates an evolutionary pressure to mutate that peptide; the more immunogenic the peptide, the higher the pressure to mutate. Conversely, we suggest that a lack of evolutionary pressure in non-immunogenic regions would result in fewer mutations and, therefore, higher homology across coronaviruses. Thus, low immunogenicity resulting in high homology can explain the low level of immune response to the high homology peptides reported here.

The reactive T-SPOT.*COVID* results in 6 of 9 asymptomatic, confirmed-infection group participants, some of whom were seronegative, demonstrate that T cells may be present in asymptomatic participants in the absence of antibodies, as reported elsewhere (Gallais et al., 2021; Schulien et al., 2020; Sekine et al., 2020).

Therefore, our finding of 2 control group participants with T-SPOT.*COVID* reactivity may be attributable to undetected asymptomatic SARS-CoV-2 infections. In support of this explanation, these participants' T-SPOT.*COVID* SFC counts of 24 and 140 were well above the 8 SFCs required for a reactive result, thus suggesting a true T cell response. The lack of response from 1 of these participants to the 5 cross-reactivity pools indicates this individual's T-SPOT.*COVID* reactivity was not due to cross-reactive T cells. The other individual had responses to 2 of the 5 cross-reactivity pools, but these responses were lower in magnitude than the response to the T-SPOT.*COVID* test, consistent with a recent SARS-CoV-2 infection and prior HCoV infections. These observations suggest that these 2 control group participants with reactive T-SPOT.*COVID* results may have had undetected, asymptomatic SARS-CoV-2 infections.

This study also showed that timing in relation to infection must be considered when interpreting immunological test results. The analysis of responses was divided into 3 time periods: 0 to 2 weeks after diagnosis, a period when the innate immune system activates the adaptive immune response (Koblischke et al., 2020; Sette and Crotty, 2021); 2+ to 8 weeks after diagnosis, when adaptive immune responses are commonly observed (Cohen et al., 2021; Tan et al., 2021); and 8+ weeks after diagnosis, when adaptive immune responses begin to taper off in some people (Cohen et al., 2021; Dan et al., 2021; Tan et al., 2021).

The percentage of T-SPOT.*COVID* reactive results in the confirmed-infection group was lowest (75.7%) within the first 2 weeks after diagnosis, as would be expected due to a 6–10+ day delay in the appearance of T cells following a SARS-CoV-2 infection (Koblischke et al., 2020; Sette and Crotty, 2021). This finding suggests that repeated testing could be appropriate in cases of a non-reactive T-SPOT.*COVID* result shortly after suspected SARS-CoV-2 exposure.

In the 2+ to 8 weeks following diagnosis, virtually all confirmed-infection group participants (98.4%) showed T-SPOT.*COVID* reactive responses indicating both the successful activation of SARS-CoV-2 reactive T cells in most infected participants and excellent detection of SARS-CoV-2-responsive T cells by T-SPOT.*COVID*.

More than 8 weeks after diagnosis, the percentage of participants with T cell responses was a little lower (83.6%), in keeping with prior reports of a reduction in T cells in some people over time. For example, migrant workers with SARS-CoV-2 infections showed a decline in SARS-CoV-2-responsive T cells over 3 months (asymptomatic) or 7 months (symptomatic) after infection (Le Bert et al., 2021). Another study reported CD4+ and CD8+ T cell responses in the blood of 70% and 93%, respectively, of COVID-19 patients at an average of 1 month post symp-

tom onset, which fell to 50% and 92% by 6+ months post symptom onset (Dan et al., 2021). A longitudinal study reported detectable SARS-CoV-2-specific T cells when measured at 10 days post symptom onset, which decreased in numbers for up to 14 weeks (Schulien et al., 2020). Taken together, these studies show that most persons with SARS-CoV-2 infection generate and retain T-SPOT.*COVID*-detectable T cells for several months after infection.

Changing T cell responses following a SARS-CoV-2 infection may explain a large percentage of the borderline results observed in our confirmed-infection group participants: borderline results could be due to the timing of the T-SPOT.*COVID* test in relationship to developing T cell responses following infection or to the falling off of T cell responses in some participants over time.

Serology showed a similar pattern of rise and fall with time, although the detection rate was lower at all time points. Overall, the T-SPOT.*COVID* test detected more confirmed-infection group participants (147 of 168) than the anti-N IgG serology test (100 of 168) (Figure 3B), a difference that was especially marked within the first 2 weeks after infection (75.7% vs 32.4%, respectively). The low level of serology responsiveness in these first 2 weeks is in keeping with the reported 2 or more weeks required to develop an antibody response (Bond et al., 2020; Tan et al., 2020, 2021), which is attributable to the need for T helper cell activation prior to antibody production and a further delayed appearance of IgG antibodies.

In participants tested more than 8 weeks after infection, the T-SPOT.*COVID* test detected more confirmed-infection group participants than serology (83.6% and 52.2%, respectively). By 20 to 36 weeks post diagnosis, T cells were detected in approximately three-quarters of the confirmed-infection group participants while anti-N IgG responses were not observed in any participants. An earlier waning of anti-SARS-CoV-2 antibodies compared with T cells, particularly anti-N antibodies, has been reported (Cohen et al., 2021; Dan et al., 2021; Schulien et al., 2020) but does not mean an ineffectual humoral response to future infections. SARS-CoV-2-responsive B cells have been detected as antibodies wane (Dan et al., 2021; Turner et al., 2021) providing a mechanism for future antibody production.

Limitations of this study include the inability to be certain that the control group participants had never been exposed to the SARS-CoV-2 virus given the endemic nature of the virus, the potential for asymptomatic infections, and the potential for false-negative test results. In addition, participants were enrolled from only one location, so these findings should be confirmed in a wider population. However, the T-SPOT.*COVID* test's hundreds of peptides could enable the test to work for most human leukocyte antigen types found worldwide. The possibility of cross-reactivity due to SARS-CoV-1 or MERS in some participants cannot be excluded; however, the possibility is low because a history of SARS-CoV-1 or MERS infections were exclusion criteria and only 27 SARS-CoV-1 and 2 MERS cases were reported in the US (CDC, 2019; WHO, 2015). In addition, no sequencing was done to determine whether any participants were infected with SARS-CoV-2 variants; however, the hundreds of SARS-CoV-2 peptides included in the T-SPOT.*COVID* test may maintain performance against SARS-CoV-2 variants. Furthermore, most known mutations have occurred in the S1 spike protein (Shah et al., 2021); therefore the T-SPOT.*COVID* nucleocapsid peptide pool could help maintain T-SPOT.*COVID* performance with SARS-CoV-2 variants.

Conclusions

In conclusion, our study demonstrated that the T-SPOT.*COVID* test effectively identified asymptomatic and symptomatic individuals with SARS-CoV-2-responsive T cells beginning shortly after infection and continuing for several months. The T-SPOT.*COVID* test identified many confirmed-infection group participants not identi-fied by anti-N IgG serology, indicating that testing for T cells in addition to antibodies can provide a more complete picture of a patient's immune response. The clinical and public health implications of a long-lasting SARS-CoV-2-specific T-cell response are unknown. Whether or not such memory T cells are protective during a future infection requires verification in prospective follow up studies.

Conflict of Interest

LT reports no conflict of interest. The remaining authors are employees of Oxford Immunotec, Ltd.

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Ethical Approval

Informed consent and study approval were obtained from Advarra IRB by NECCR at Primacare.

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