Clinical Validation of a Novel T-cell Receptor Sequencing Assay for Identification of Recent or Prior SARS-CoV-2 Infection

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RUNNING TITLE

COVID-19 T-cell Clinical Test Validation

ABSTRACT

Background

While diagnostic, therapeutic, and vaccine development in the COVID-19 pandemic has proceeded at unprecedented speed, critical gaps in our understanding of the immune response to SARS-CoV-2 remain unaddressed by current diagnostic strategies.

Methods

A statistical classifier for identifying prior SARS-CoV-2 infection was trained using >4000 SARS-CoV-2–associated TCRβ sequences identified by comparing 784 cases and 2447 controls from 5 independent cohorts. The T-Detect™ COVID assay applies this classifier to TCR repertoires sequenced from blood samples to yield a binary assessment of past infection. Assay performance was assessed in 2 retrospective (n=346; n=69) and 1 prospective cohort (n=87) to determine positive percent agreement (PPA) and negative percent agreement (NPA). PPA was compared to 2 commercial serology assays, and pathogen cross-reactivity was evaluated.

Results

T-Detect COVID demonstrated high PPA in individuals with prior RT-PCR–confirmed SARS-CoV-2 infection (97.1% 15+ days from diagnosis; 94.5% 15+ days from symptom onset), high NPA (~100%) in presumed or confirmed SARS-CoV-2 negative cases, equivalent or higher PPA than 2 commercial serology tests, and no evidence of pathogen cross-reactivity.

Conclusion

T-Detect COVID is a novel T-cell immunosequencing assay demonstrating high clinical performance for identification of recent or prior SARS-CoV-2 infection from blood samples, with implications for clinical management, risk stratification, surveillance, and understanding protective immunity and long-term sequelae.

KEYWORDS

SARS-CoV-2, T-cell receptor, next-generation sequencing, diagnostic, COVID-19

INTRODUCTION

Knowledge gaps in our understanding of immunity to SARS-CoV-2 infection translate into critical areas of unmet need in diagnosis and management of COVID-19 and epidemiologic monitoring of the pandemic. Serologic testing of IgM, IgG, and/or IgA isotypes has been the primary modality for identifying prior SARS-CoV-2 infection, estimating disease prevalence, and evaluating immunity [1,2]. Although antibody testing has been shown to capture a larger percentage of exposures than polymerase chain reaction (PCR) testing [3], it is limited by interassay variability [4]; low or absent antibody titers in individuals with asymptomatic or mild infection [5,6]; declining antibody levels over time [7]; and false-positive results [1]. It also remains unclear whether serology results correlate with long-term protective immunity or prevention of transmission, especially in light of evidence that vaccinated individuals can transmit viral variants [2,8]. Finally, serologic testing may not reflect the full extent of pre-existing immunity, as SARS-CoV-2–reactive T-cells have been identified in 20%–50% of individuals with no known exposure [9].

Humoral responses vary among vaccinated or exposed individuals, and 5%–20% of individuals recovered from SARS-CoV-2 infection may have no detectable antibodies, depending on isotype and disease severity [10–12]. Multiple lines of evidence support a central role of the cellular response in SARS-CoV-2 immunity [13,14]. The majority of patients diagnosed with COVID-19, including convalescent patients presenting across a wide spectrum of disease severity, generate $CD8^+$ and $CD4^+$ T-cell responses [9,15,16], which have been associated with milder disease and protection from infection [17,18]. T cells also play a critical role in activating the humoral response and can precede antibodies as the first detectable immune response to SARS-CoV-2 infection, particularly in asymptomatic or mild illness [15]. SARS-CoV-2–specific T

cells are persistent, remaining elevated at least 1 year post infection, in some cases in the absence of seroconversion [13,19–21]. Additionally, the observation that some emerging variants of concern (VOCs) evade antibody responses while largely preserving the T-cell response [16,22] underscores the critical importance of understanding the resulting effects on infectivity and vaccine-induced immunity.

Features inherent to the T-cell response make it a desirable target for identifying and tracking disease exposure. The cellular immune response is sensitive, antigen-specific, and is amplified through expansion of clones that circulate in the blood and are maintained in long-term memory. Here we describe the implementation and clinical validation of T-Detect™ COVID, a novel high-throughput assay that has received Emergency Use Authorization (EUA) for determining recent or prior SARS-CoV-2 infection based on T-cell receptor gene sequencing and subsequent repertoire profiling from whole blood samples [23]. We demonstrate high positive percent agreement (PPA) and negative percent agreement (NPA) in PCR-confirmed SARS-CoV-2 cases across several cohorts and longitudinal timepoints. This assay has equivalent or better performance than commercially available EUA antibody tests and lacks cross-reactivity to several respiratory pathogens.

METHODS

Ethics

All samples were collected pursuant to an Institutional Review Board-approved clinical study protocol. For residual samples collected under prospective study protocols (see Supplemental Methods), informed consent was obtained from participants. All other samples were collected as clinical remnants. The implementation and clinical validation of T-Detect COVID was

conducted following US Food and Drug Administration guidance "*Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) May 2020."*

Clinical Cohorts and Sample Allocation

Clinical specimens were collected via a retrospective arm that included SARS-CoV-2–positive and –negative residual samples from prior research studies and remnant clinical samples and a prospective arm that included samples from participants with symptoms compatible with COVID-19 who tested either positive or negative by SARS-CoV-2 reverse transcription (RT)-PCR. Development of the T-Detect COVID classifier included cross-validation using the training sample set and secondary validation using a holdout sample set. The training and holdout sample sets included confirmed SARS-CoV-2 positive cases, as well as negative controls. Additional details regarding the case and control cohorts used for classifier development and validation (training and holdout sets) are provided in Supplemental Tables 1 and 2 and in the Results section.

For the PPA and NPA clinical validation studies, samples from both arms were analyzed using the T-Detect COVID assay (Tables 1 and 2). A detailed description of the allocation plan and study cohorts is included in Supplemental Methods. When available, paired serum samples from cohorts used for secondary PPA analyses (Table 1; n=77) were tested using 2 different EUA antibody assays: 1) Elecsys® Anti-SARS-CoV-2; Roche (all isotypes); and 2) SARS-CoV-2 Antibody, IgG; LabCorp (see Supplemental Methods for details).

Classifier Development and Training

Public TCRs may result from exposure to a common antigen and are more common among those with shared human leukocyte antigen (HLA) backgrounds [24,25]. Similar to the approach

described in a previous study [19], this phenomenon was leveraged to create a predefined list of public, rearranged TCRβ amino acid sequences significantly enriched among confirmed SARS-CoV-2–positive cases compared to controls. Briefly, 1-tailed Fisher's exact tests were performed on all unique TCR sequences to identify sequences exclusive to, or greatly enriched in, SARS-CoV-2 PCR–positive samples (n=784) versus negative controls (n=2447). These public sequences served as the basis for using a machine-learning algorithm to train a classifier capable of identifying patients with an immune response to SARS-CoV-2. The classifier was created by logistic regression with 2 dependent variables: the number of unique TCRβ DNA sequences encoding a SARS-CoV-2–associated sequence and the total number of unique TCRβ DNA sequences in the sample. A 5-fold cross-validation of the training set was used to identify the final *P*-value cutoff in the 1-tailed Fisher's exact test that yielded the optimal area under the receiver operating characteristic (AUROC) and was also used to evaluate assay performance with the training data. To further refine the method described in our previous study [19], the diagnostic model threshold was set to 99.8% specificity against an independent holdout set of 1657 negative controls and 100 positive controls.

T-Detect COVID Assay

Process Overview

T-Detect COVID consists of a core assay designed to sequence and quantify rearranged TCRβ sequences from genomic DNA (gDNA) extracted from peripheral blood and diagnostic software that applies the COVID-specific algorithm to the TCRβ sequence repertoire data to determine a result. An overview of the assay is shown in Figure 1 and described below.

Sample Collection and Processing

Peripheral whole blood is collected in a 10 -mL ethylenediaminetetraacetic acid (EDTA) vacutainer tube and shipped overnight at ambient temperature to the Adaptive Biotechnologies' clinical laboratory. Upon receipt, the sample is accessioned and stored at 4°C for processing that same day via automated gDNA extraction or stored at –80°C for later extraction.

Sample and Library Preparation, Sequencing, and Pipeline Analysis

Detailed methods for sample preparation, immunosequencing, and pipeline analysis have been described [19,26]. Briefly, a target gDNA sample input of 18 µg is isolated from 2 mL fresh or frozen peripheral whole blood (6 mL requested). This target gDNA input ensures that samples meet the minimum unique productive rearrangements input quality control (QC) specification. A multiplex PCR strategy with control synthetic TCRβ molecules added to each reaction is used to amplify rearranged TCRβ sequences from gDNA. PCR libraries are loaded together on a single sequencing run, and sequencing is performed using the Illumina NextSeq 500/550 System. Sequence data are extracted, and reads are attributed to data derived from biological versus synthetic templates to calculate template estimates for each identified receptor sequence, as well as input cell counts.

T-Detect COVID Algorithm

The SARS-CoV-2–specific algorithm (classifier) is applied to the core assay output for each sample to make the COVID-positive/negative call based on the resulting score. As described above, the classifier identifies and quantifies any SARS-CoV-2–associated TCRs from a predetermined list of several thousand SARS-CoV-2–associated TCRs, and it also quantifies all unique non-SARS-CoV-2 TCRs identified. These two variables were used in the machine

learning classifier to produce the final score for each sample. The total number of unique TCR sequences must fall within a threshold for the algorithm to produce a valid result. The pre-specified threshold is then applied to classify the patient sample as positive or negative for an immune response to SARS-CoV-2. The classifier was locked to create the COVID-specific algorithm for T-Detect COVID prior to clinical validation.

RESULTS

Public, SARS-CoV-2–Associated Enhanced Sequences (ESs) Distinguish Cases From Controls

We began with the previously described classifier [19], which was trained on 784 SARS-CoV-2–positive cases (from 5 independent cohorts, detailed in Supplemental Table 1) and 2448 controls (from 4 independent cohorts, detailed in Supplemental Table 1). Adjustment of the training set to 2447 controls yielded a final total of 4469 unique SARS-CoV-2–specific enhanced sequences. In order to further validate the classifier for clinical use, we tested it on a new independent holdout set of 100 cases and 1657 controls (Figure 2 and Supplemental Tables 1 and 2). We evaluated performance of the classifier in samples stratified by age and sex via both 5-fold cross-validation of the training data and using the independent holdout set described above. Performance of the classifier was generally robust to age and sex, although age was weakly associated with COVID score, likely because disease severity is known to vary by age [27] (Figure 3 and Supplementary Table 3). SARS-CoV-2–associated sequences varied substantially in terms of publicity, with the rarest sequences appearing in just 5 cases and no controls and the most public appearing in 265 cases and 322 controls (Supplementary Figure 1). We set the diagnostic model threshold to 99.8% specificity in the 1657 negative controls from the independent holdout set (Figure 2).

Independent studies conducted in parallel suggest that the sensitivity of T-cell testing is equivalent to or better than that of serologic assays [28,29].

While developing a classifier for Lyme disease, we found that incorporating additional sequences that did not meet strict enrichment thresholds but showed other evidence of disease association, such as shared sequence similarity with a Lyme-associated sequence and/or elevated clonal abundance, improved classifier performance [30]. However, addition of sequences with other evidence of disease association did not improve classification sensitivity of the SARS-CoV-2 classifier, likely because the SARS-CoV-2 classifier is already sensitive enough to detect the vast majority of COVID-19 cases with an appreciable T-cell response. Thus, these sequences were not included in the final model.

High PPA With SARS-CoV-2 PCR

Two PPA studies were undertaken to evaluate T-Detect COVID assay performance in individuals with PCR-confirmed SARS-CoV-2 using independent samples that were not used in classifier development or in the holdout set. The primary PPA analysis considered days since diagnosis, and the secondary PPA analysis considered days from symptom onset. In the primary PPA study, 205/222 samples from the DLS cohort (but distinct from the samples used for classifier development) passed all QC and threshold requirements, making them eligible for analysis (Table 1). In the secondary PPA study, all 77 independent samples tested (from the ImmuneRACE and ImmuneSense COVID-19 cohorts) were from unique individuals, passed QC and threshold requirements, and were included for analysis (Table 1). Samples were collected a maximum of 106 days from symptom onset. PPA for the T-Detect COVID assay was highest (97.1%) ≥15 days since diagnosis in the primary analysis and ≥15 days since symptom onset (94.5%) in the secondary analysis (Table 3).

High NPA in Presumed and/or Confirmed SARS-CoV-2 Negative Samples

Two NPA studies were undertaken to evaluate T-Detect COVID assay performance: a primary NPA analysis of retrospectively sourced whole blood samples from pre-pandemic timepoints (July 2017–Nov 2019; independent samples belonging to the DLS cohort) presumed to be SARS-CoV-2 negative and a secondary NPA analysis of samples prospectively collected from symptomatic individuals in the ImmuneSense COVID-19 cohort who tested negative for SARS-CoV-2 (Table 2). In the primary NPA study, 87 of 124 samples were from unique individuals, passed all standard QC and assay threshold requirements, and were included for analysis, yielding an NPA of 100% (Table 4). The majority of excluded samples failed to meet assay QC criteria or assay-specific thresholds, which may have been due to the variable collection conditions and biological/disease context associated with retrospective sourcing. The secondary NPA study assessed T-Detect COVID assay performance prospectively in samples from individuals presenting with compatible symptoms who tested negative for SARS-CoV-2 by RT-PCR (BioFire RP2.1 EUA) and EUA antibody tests. Of 79 individuals meeting these criteria, no samples failed QC or performance thresholds, and all samples were included for analysis, yielding an NPA of 98.7% (Table 4).

Equivalent or Greater PPA Than EUA Antibody Tests in Confirmed SARS-CoV-2 Cases

Additional analyses compared the PPA of the T-Detect COVID assay with that of antibody testing in paired SARS-CoV-2–positive samples from 77 individuals (used in secondary PPA analyses). Results of these analyses showed that the PPA for T-Detect COVID was as high or higher than that of serology, particularly in the early phases of infection (Table 5).

Lack of Cross-Reactivity with Other Viruses/Pathogens

Development of the classifier for this assay leveraged the biology of the T-cell–mediated response to infection, which requires an inherent specificity between the TCRs in SARS-CoV-2–infected patients and the cognate antigens unique to SARS-CoV-2. Assay specificity had been further refined by establishing the clinical call threshold at 99.8% in 1657 controls (known negative samples) collected in the United States prior to December 2019, a population characterized by a high prevalence of vaccinated or natural immunity to potentially cross-reactive viruses. Specificity was further verified in a set of blood and peripheral blood mononuclear cell (PBMC) samples (n=38) collected from individuals infected with influenza A/B, *Haemophilus influenzae b*, human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) to assess potential cross-reactivity. None of these samples tested positive using the T-Detect COVID assay (Table 6).

DISCUSSION

Despite the critical role of both humoral and cellular immune responses in SARS-CoV-2 infection, serologic testing is the predominant method for assessing previous infection, population-level prevalence, and potential immunity. It offers advantages of relatively low cost, fast turnaround, and scalability [31]. However, limitations of SARS-CoV-2 serologic testing, such as variability in performance across platforms and antibody isotypes, waning antibody signal over time [4,7], and absence of detectable antibodies in 5%–20% of individuals [11,12], expose unmet clinical and public health needs for complementary testing strategies.

In this study, we describe a TCR sequence-based assay for identifying recent or prior SARS-CoV-2 infection in whole blood samples that demonstrates high PPA in confirmed SARS-CoV-2–positive samples (>97% beyond 15 days following diagnosis), high NPA in presumed or confirmed negative samples (~100%), equivalent or higher PPA compared to commercially

available EUA serology tests, and lack of cross-reactivity with multiple viral and/or respiratory tract pathogens. Assay performance was consistent across several retrospective and prospective cohorts and longitudinal sampling timeframes. SARS-CoV-2–specific T-cell responses have been shown to persist at least 1 year [13,20], and application of our approach in a real-world setting showed evidence for robust and durable T-cell signals [28].

In contrast to functional T cell-based assays, including ELISpot, T-Detect COVID can identify antigen-specific T cells irrespective of their functional status. We have previously shown that for T cells with specificity for other viral pathogens, ELISpot and the Multiplexed Identification of TCR Antigen (MIRA) assay exhibit a high degree of correlation [32]. More recently, our COVID-19 classifier showed a positive correlation with MIRA [19]. An obvious advantage of the T-Detect COVID assay is that it includes a dynamic learning system facilitated by constant additions of TCR sequences from COVID-19 samples to the database. In addition, our immune medicine platform has the ability to identify exposure to multiple pathogens, simultaneously providing a broader picture of the adaptive immune system. Finally, we previously reported a direct correlation between the magnitude of the measured SARS-CoV-2 T-cell response (in depth and breadth) and prior disease severity [28,29].

These observations support the clinical utility of T-cell profiling for risk stratification, detection of remote prior infection, informing public health and surveillance strategies, and clarifying correlates of immune protection by providing a more comprehensive characterization of the immune response to SARS-CoV-2. Our diagnostic immunosequencing platform has the potential to yield clinical insights across multiple disease states [26]. Furthermore, robust T-cell profiling can also inform vaccine development. Vaccines targeting SARS-CoV-2 are capable of inducing type 1 helper T-cell (Th1) responses, in addition to antibodies that decline over time [9,33].

Thus, a combination of serologic testing and T-cell repertoire profiling can enable broader characterization of the immune response to SARS-CoV-2 vaccination.

Limitations of our study include small samples sizes in some cohorts (<15 days post symptom onset), very limited data from pediatric patients, and the lack of availability of other seasonal human coronavirus (HCoV) samples for cross-reactivity analysis, as blood is not commonly drawn in the clinical diagnosis or treatment of these viruses. Notably, >98% of individuals display antibodies against 3 of the 4 common HCoV strains [34]. Therefore, a significant number of our controls would be expected to have immune responses against HCoVs, adding confidence to the specificity of our TCR signal. Lastly, the sample size in the prospective ImmuneSense collection cohort, in particular COVID-19 cases, is limited.

T-Detect COVID is the first TCR sequencing-based assay for interrogation of the cellular immune response in SARS-CoV-2, and our results show that the assay demonstrates ≥95% positive agreement in identifying prior exposure/infection with ~100% negative agreement and equivalent or higher performance than commercial EUA serologic testing. The assay can provide critical insights into disease pathogenesis, severity, recovery, and immune protection. Future studies will help establish the merits of this approach for immunology research, vaccine/drug development, and public health/surveillance strategies.

NOTES

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Potential conflicts of interest. SCD declares employment and equity ownership with Adaptive Biotechnologies and employment with Stanford University School of Medicine at the time of this study. TM and LB declare leadership, employment, and equity ownership with Adaptive Biotechnologies at the time of this study. All other authors declare employment and equity ownership with Adaptive Biotechnologies at the time of this study.

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Table 1. Description of RT-PCR–Positive SARS-CoV-2 Samples Used for Primary & Secondary PPA Analyses

	Primary Analyses	Secondary Analyses	
Cohort name	Discovery Life Sciences (DLS)[a]	ImmuneRACE[a]	ImmuneSense™ COVID-19[a]
Detailed cohort information	Retrospective clinical remnant samples from individuals positive for SARS-CoV-2	Retrospective use of residual samples from a prior research study with confirmed SARS-CoV-2 infection via medical record search (NCT04494893)	Prospective collection of individuals being tested for SARS-CoV-2, included participants that tested positive for SARS-CoV-2 (NCT04583982)
Number of unique samples	222	69	8
Study population	Basic demographics, from a New York	Enrolled ages 18-89, samples collected nationwide,	Enrolled ages 18-89, two clinical drive-through testing sites

	reference lab	24 virtual locations throughout the US	in New Jersey
Sample types	Frozen whole blood	Frozen whole blood	Frozen whole blood
RT-PCR comparator test	Abbott RT-PCR SARS-CoV-2 EUA	Multiple independent EUA test methods	Abbott RT-PCR SARS-CoV-2 EUA

Abbreviations: COVID-19, coronavirus disease 2019; EUA, Emergency Use Authorization/Authorized; PPA, positive percent agreement; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]A detailed description of these cohorts is provided in the Supplement.

Table 2. Description of SARS-CoV-2–Negative Samples Used for Primary and Secondary NPA Analyses

	Primary NPA	Secondary NPA
Cohort name	Discovery Life Sciences (DLS)[a]	ImmuneSense COVID-19[a]
Cohort details	Retrospective collection	Prospective collection
Number of unique negative samples	124	79
Study population	Diverse populations collected pre–COVID-19 within the United States upon presentation to clinic with a variety of symptoms, including respiratory illnesses	Single site collection, New Jersey
Dates of collection	Jul. 2017–Nov. 2019	Oct.–Dec. 2020

Sample types	Frozen blood	Frozen blood
Nasopharyngeal test		Abbott RT-PCR SARS-CoV-2 EUA
Comparators test at time of collection		BioFire RP 2.1 EUA
Antibody test comparators at time of collection		Abbott Architect SARS-CoV-2 IgG Roche Elecsys Anti-SARS-CoV-2

Abbreviations: COVID-19, coronavirus disease 2019; EUA, Emergency Use Authorization/Authorized; IgG, immunoglobulin G; NPA, negative percent agreement; RP, respiratory pathogen; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]A detailed description of these cohorts is provided in the Supplement.

Table 3. PPA of the T-Detect COVID Assay With SARS-CoV-2 RT-PCR According to Days Since Symptom Onset or Days Since Diagnosis

Days Since Diagnosis	RT-PCR–Positive Samples (n)	T-Detect–Positive Samples (n)	T-Detect PPA (95% CI)
0–7 days	35	25	71.4 (53.7–85.4)
8–14 days	33	31	93.9 (79.8–99.3)
≥15 days	137	133	97.1 (92.7–99.2)
All (range, 0–91 days)	205	NA	NA
Days Since Symptom Onset			
0–7 days	13	7	53.8 (25.1–80.8)
8–14 days	9	7	77.8 (40.0–97.2)
≥15 days	55	52	94.5 (84.9–98.9)
All (range, 0–106 days)	77	NA	NA

Abbreviations: CI, confidence interval; COVID/COVID-19, coronavirus disease 2019; NA, not applicable; PPA, positive percent agreement; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4. NPA of the T-Detect COVID Assay With Pre-pandemic Samples Sourced Retrospectively (DLS) and Prospectively Enrolled Participants (ImmuneSense COVID-19) Negative for SARS-CoV-2 by EUA RT-PCR and Antibody Testing

Cohort	Samples (n)	T-Detect–Negative Results (n)	NPA (95% CI)
DLS	87	87	100 (95.8–100)
ImmuneSense COVID-19	79	78	98.7 (93.1–99.97)

Abbreviations: CI, confidence interval; COVID/COVID-19, coronavirus disease 2019; DLS, Discovery Life Sciences cohort; EUA, Emergency Use Authorization/Authorized; NPA, negative percent agreement; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 5. PPA of T-Detect COVID Assay Results Compared to Serology-Based Assays in Paired Samples

Days Since Symptom Onset	Samples (n)	T-Detect COVID PPA (95% CI)	Abbott Architect SARS-CoV-2 IgG PPA (95% CI)	Roche Elecsys Anti–SARS-CoV-2 PPA (95% CI)
0–7	13	53.8 (25.1–80.8)	15.4 (1.9–45.4)	15.4 (1.9–45.4)
8–14	9	77.8 (40–97.2)	22.2 (2.8–60)	22.2 (2.8–60)
≥15	55	94.5 (84.9–98.9)	88 (75.7–95.5)	90.4 (79–96.8)

Abbreviations: CI, confidence interval; COVID/COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; PPA, positive percent agreement; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 6. T-Detect COVID Assay Results Indicating 100% Specificity (Lack of Cross-reactivity) in Individuals Infected With Influenza A/B, *H. influenza b*, HIV, HCV and/or HBV

Infectious Agent	Samples (n)	Source/Type	T-Detect Assay Positives (n)
Influenza A	11	Whole blood	0
Influenza B	11	Whole blood	0
Haemophilus influenzae b	3	Whole blood	0
HIV	5	Frozen PBMCs	0
HCV	7	Frozen PBMCs	0
HBV	1	Frozen PBMCs	0

Abbreviations: COVID/COVID-19, coronavirus disease 2019; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cells.

FIGURE LEGENDS

Figure 1. T-Detect COVID assay process overview. Overview of steps involved in sample analysis used for clinical validation of T-Detect COVID. Following development and analytical validation (described in Figures 2 and 3), the classifier was locked and used for analysis of independent clinical samples (PPA/NPA as well as cross-reactivity testing). Schematic depicts major steps involved in sample acquisition, sample processing, data analysis, and scoring to determine SARS-CoV-2 positive/negative status, including additional detail related to the distinct steps of the core T-Detect COVID assay.

Abbreviations: COVID, coronavirus disease 2019; gDNA, genomic DNA; NPA, negative percent agreement; PPA, positive percent agreement; TCRβ, T-cell receptor beta.

Figure 2. The T-Detect COVID classifier incorporates enhanced sequences associated with SARS-CoV-2 infection and can distinguish cases from controls. A training set of 784 confirmed SARS-CoV-2–positive cases (from the DLS, NIH/NIAID, ISB, H12O, and BWNW cohorts) and 2447 controls (from the DLS, Bay Area Lyme, OHSU, and Hôpital Saint-Louis cohorts) resulted in the identification of 4469 enhanced TCR amino acid sequences that formed the basis for the classifier, which successfully distinguished cases from controls in the training dataset. The classifier was then applied to an independent holdout set of 100 confirmed SARS-CoV-2–positive cases (from ImmuneRACE cohort) and 1657 controls (from cohorts including Johns Hopkins Lyme, FHCRC cancer, Moffitt pancreatic cancer, and independent DLS samples). Supplemental Table 1 provides additional cohort details.

Abbreviations: BWNW, Bloodworks Northwest (Seattle, WA); CDR3, complementarity-determining region 3; COVID, coronavirus disease; DLS, Discovery Life Science; FHCRC, Fred

Hutchinson Cancer Research Center; H12O, Hospital 12 de Octubre; ISB, Institute of Systems Biology Covid-19 Immune Response Study; J, joining gene; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; OHSU, Oregon Health and Science University; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCR, T-cell receptor; V, variable gene.

Figure 3. Performance of T-cell classifier to separate SARS-CoV-2 cases from controls is consistent across age and gender. Plots depict analyses of the classifier training and holdout sample sets used in Figure 2, stratified by age and sex of donors. Performance of T-cell classifier to separate SARS-CoV-2 cases from controls is consistent across age groups (A, C) and in both males and females (B, D). Both plots report model scores as the untransformed log-odds estimated from the logistic regression classifier in training cross-validation (A, B) and in an independent holdout set (C, D). The violin plots in panels (B) and (D) visualize the density of log-odds scores among male and female cases and controls, with median and interquartile range values indicated.

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

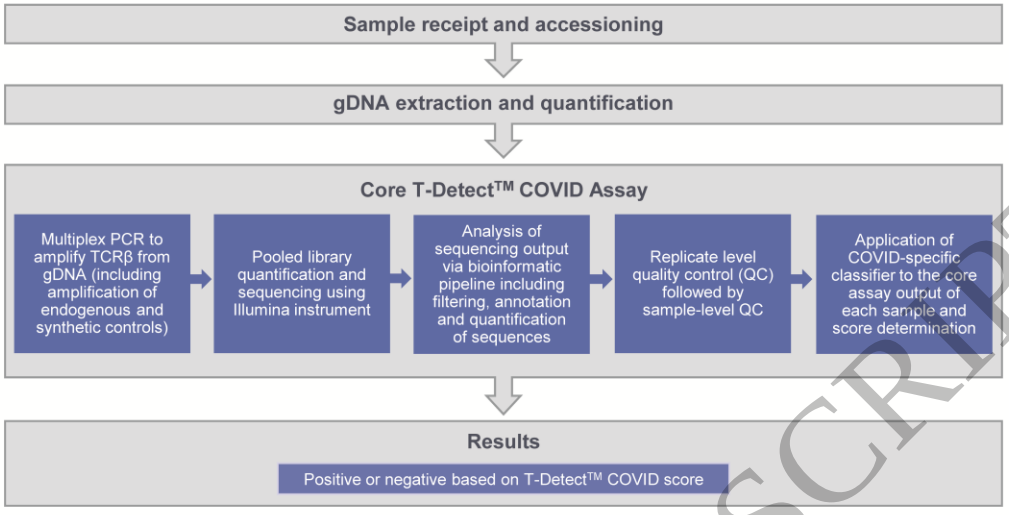

Figure 1
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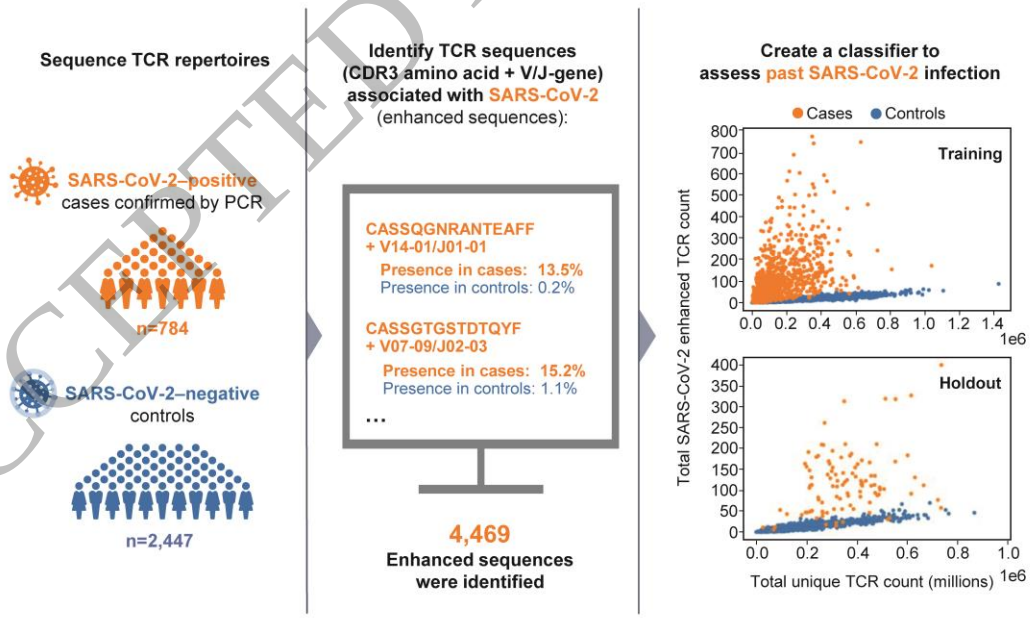

Figure 2
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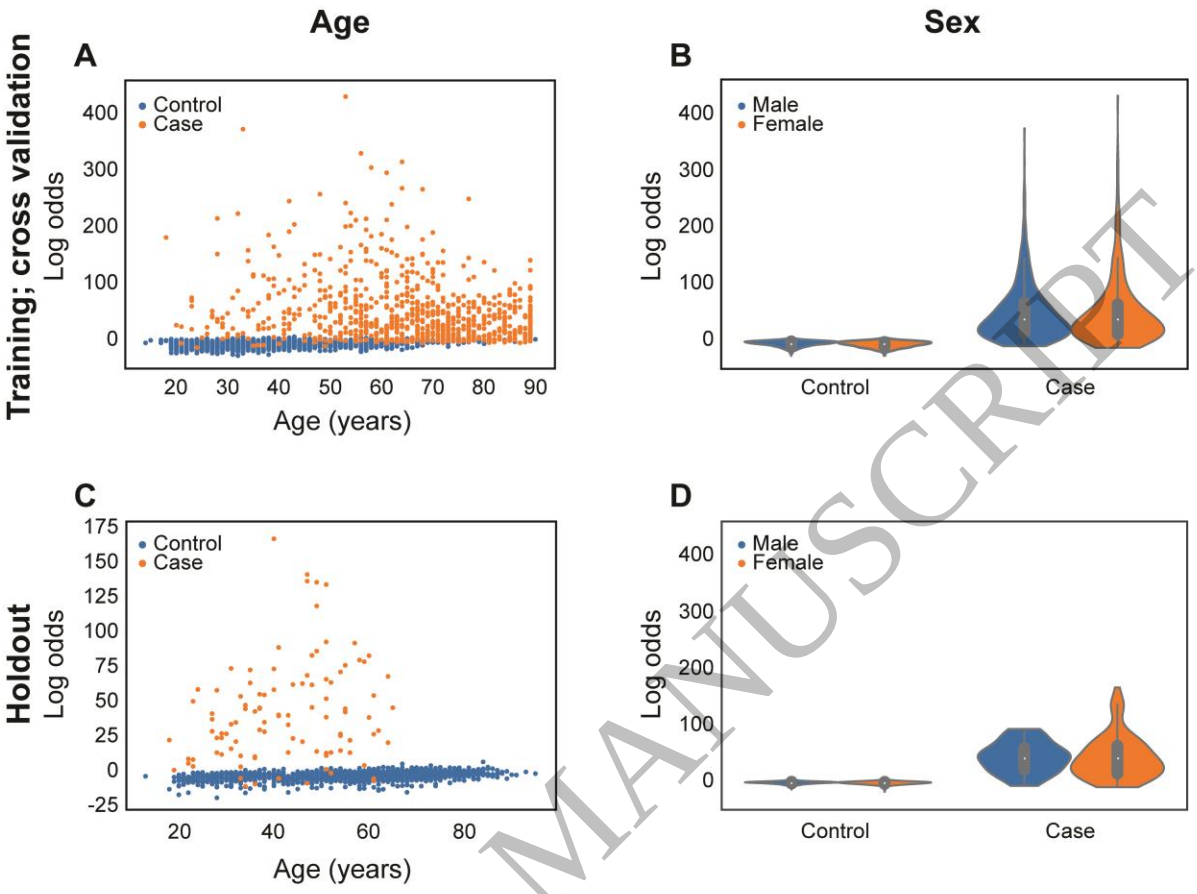

Figure 3
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