EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
T-Detect COVID Test
Adaptive Biotechnologies Corporation

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

The T-Detect COVID Test will be performed at laboratories designated by Adaptive Biotechnologies Corporation that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests as set forth in this EUA.

INTENDED USE

1) Intended Use:

The T-Detect COVID Test is a multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) based assay to detect and identify rearranged T-cell receptor beta (TCR β) gene sequences from human genomic DNA (gDNA) isolated from venous whole blood using di-potassium ethylenediaminetetraacetic acid (K2 EDTA) as an anticoagulant. The T-Detect COVID Test is intended for use as an aid in identifying individuals with an adaptive T-cell immune response to SARS-CoV-2, indicating recent or prior infection with SARS-CoV-2. At this time, it is unknown how long the T-cell immune response persists following infection and what level of protection may be conferred by the presence of a T-cell immune response. The T-Detect COVID Test should not be used to diagnose or exclude acute SARS-CoV-2 infection. Testing is limited to laboratories designated by Adaptive Biotechnologies Corporation that includes the Adaptive Biotechnologies Lab located at 1551 Eastlake Ave E Ste 200, Seattle, Washington, which is also certified under the Clinical Laboratory Improvements Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets requirements to perform high-complexity tests.

Results are for identification of specific T-cell receptor beta (TCR β) gene sequences specific for SARS-CoV-2 from human gDNA. It may take several days after initial infection to prime and expand adaptive T cell immune responses, although the duration of time the adaptive T cell immune responses are present is not well characterized for SARS-CoV-2 infections.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of T-Detect COVID Test early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing using a FDA approved, cleared, or authorized molecular or antigen test for SARS-CoV-2 is necessary. The results from the assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Specimens should only be tested from individuals that are 15 days or more post-symptom onset.

Incorrect results for T-Detect COVID Test may occur due to biologic variation of the T-cell receptor (TCR) repertoire or other possible causes.

Testing with the T-Detect COVID Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the use of the Illumina NextSeq 500 and NextSeq 550 Sequencing Systems and Next-Generation Sequencing workflows. The T-Detect COVID Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TESTPRINCIPLE

The T-Detect COVID Test is a targeted NGS approach that sequences and quantifies rearranged TCR β sequences from gDNA extracted from venous whole blood. The assay utilizes a machine learning algorithm to identify patients with an immune response to SARS-CoV-2 based on the observed rearranged TCR β sequences. A pre-defined list of rearranged TCR β sequences associated with patients that have being previously identified as SARS-CoV-2 positive is used in the machine learning algorithm to identify patients with an immune response to SARS-CoV-2.

Venous whole blood should be collected into a 10mL K2-EDTA vacutainer tube and shipped to the testing site at ambient temperature in a protected sample shipper. The blood samples are stored under the following conditions until gDNA extraction is performed.

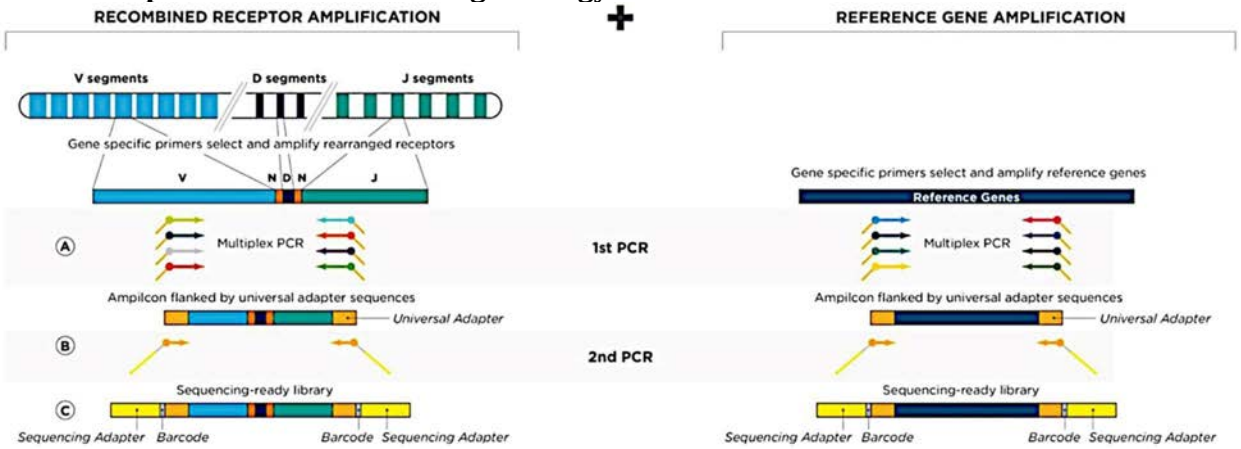
Table 1. Blood Sample Storage Condition

Storage Temperature	Storage Time
Ambient (15°C-30°C)	5 days
Refrigerated (0°C-8°C)	14 days
Frozen (-15°C - -25°C)	6 months
Frozen (-90°C - -70°C)	12 months

DNA extraction from blood sample is performed using the Kingfisher Flex 711 system and the MagMax DNA Multi-sample Ultra 2.0 reagent kits. A minimum of 18 µg of gDNA is extracted from at least 2 mL of blood sample.

Genomic DNA extracted from each biological sample is split into 4 replicate reaction wells for PCR amplification with synthetic TCR β molecules added to each reaction. Because a known number of synthetic molecules are added to each PCR reaction, these molecules are used to calculate the absolute quantity of any given sampled TCR β sequence. A multiplex PCR strategy is used to amplify rearranged TCR β sequences from gDNA, reference loci, synthetic TCR β molecules, and synthetic reference molecules (see Figure 1 below).

Figure 1. Multiplex PCR and Barcoding Strategy



Sets of primers are used to target the V and J gene segments of the human TCR β gene; the V and J gene specific primers include a 5' universal adapter sequence. Amplicons are generated from rearranged TCR β genes (and synthetic TCR β molecules) and cover the CDR3 region, as well as some adjoining V and J gene segment sequence. The universal adapters on the flanks of the first amplicon serve as priming sites in a second PCR. The second PCR primers add well-specific barcode sequences around the amplicon and adapters that allow for sequencing by synthesis. In parallel, reference loci (from gDNA and synthetic molecules) are amplified with several pairs of gene-specific primers, using the same adapter and barcoding strategy, resulting in amplicons of similar size to a typical rearranged TCR β amplicon. Data from all PCR replicates are included in the final output. Comparisons between PCR replicates are used both for quality control and to improve quantitation.

The pooled library product is quantified with the KAPA Library Quantification Kits using a q-PCR based library quantification method. Pooled libraries of up to 48 samples (including controls) are pooled and loaded together on a single sequencing run. Sequencing is conducted with the Illumina NextSeq 500/550 System using the Illumina NextSeq500/550 High Output Kit v2 (150 cycles). The sequencing instruments are run using dual-indexed paired-read sequencing setup with 156 cycles for read-1 and 12 cycles for read-2. The samples are also sequenced with indexed PhiX library control to provide sufficient sequence diversity, which assists with template registration and improves run and base call quality.

Sequencing output from the pooled library is first analyzed using the core assay data pipeline. The core assay data pipeline first processes the instrumental data generated by the NextSeq 550/500 sequencer using Illumina supplied software to convert the raw instrumental data into text files containing base calls and quality scores. Reads are then split into sample replicates according to replicate specific barcodes in the read data and each replicate is then analyzed. For each replicate, primers and adapter sequences are removed during data processing, and the reads are trimmed to a uniform length. Based on the sequences carried by the synthetic templates, reads are split into data derived from biological templates and data derived from synthetic templates. The data derived from synthetic templates is used, along with biological sequence read counts, to derive template estimates for each identified receptor sequence as well as estimate input cell counts. The reads derived from biological templates are clustered in order to account for sequencing error, after which a final filtering step is performed. After filtering, the sequences are annotated, and then quantified and replicate level Quality Control (QC) is performed. After each replicate is processed, replicates originating from the same sample are combined and sample level QC is performed.

The COVID-specific algorithm (classifier) is applied to the core assay output of each sample and generates the COVID positive/negative result for each sample. The classifier identifies and quantifies any SARS-CoV-2-associated T-Cell Receptor Sequences (TCRs) from a predetermined list of several thousand SARS-CoV-2 associated TCRs and also quantifies all the unique TCRs identified. These factors are mathematically combined into a score representing the relative enrichment for SARS-CoV-2-associated TCR β sequences. A pre-specified threshold is then applied to classify the patient sample as positive or negative for an immune response to SARS-CoV-2. The T-Detect COVID Test patient report is generated after algorithm results are combined with the patient data necessary to render the final clinical report.

INSTRUMENTS USED WITH THE TEST

The T-Detect COVID Test is to be used with the following instruments:

Table 2A. Instruments for Use with the T-Detect COVID Test

Instrument	**Manufacturer**	**Catalogue #**
Thermo Scientific Kingfisher Flex 711	Thermo Scientific	5400610
Bio-Rad C1000 w/96W Deep Thermal Cycler Block	Bio-Rad	185-1096
Bio-Rad CFX384 Real-Time PCR Detection System qPCR Detection System 3.1	Bio-Rad	185-5384
Eppendorf MixMate	Eppendorf	5353000529
Illumina NextSeq 500 or 550 Sequencer	Illumina	SY-415-1001 SY-415-1002
Hamilton Robotics STAR or STARlet Liquid Handling Robot	Hamilton	
VisionMate tube barcode reader	VisionMate	3125
NextSEQ Control Software v4.0.1	Illumina	N.A.
Adaptive Analysis Pipeline Software (Assay SW)	Adaptive	N.A.

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the T-Detect COVID Test IFU and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized IFU, which includes the instrument qualification protocol, as per the letter of authorization.

CONTROLS TO BE USED WITH THE TEST

1. **DNA Extraction controls:** Each batch extraction (up to 22 samples) is performed with one positive control (Whole Human Blood Frozen with 10% DMSO) and one negative extraction control (Molecular Grade Water). The extraction negative control is used to confirm lack of contamination during the extraction process and is processed through amplification and sequencing in the same fashion as samples. The positive control is included to assess the effectiveness of the extraction process. The negative DNA Extraction control is processed the same as a regular sample and is subject to the core assay quality control specifications. A failure in one of the extraction controls requires further assessment by the clinical laboratory director (CLD) to determine if a repeat of the extraction process is required for one or all samples. Alternatively, all samples may be failed leading to re-extraction.

2. **TCR β synthetic templates:** Each PCR reaction includes a spike-in of synthetic molecules that mimic biological TCR β sequence amplicons. The synthetic TCR β molecules allow for measurement and correction of residual amplification bias. Because a known number of synthetic molecules are added to each PCR reaction, these molecules are used to calculate the absolute quantity of any given sampled TCR β sequence.

3. **Non-TCR β control loci:** Each PCR reaction includes primer sets to amplify control loci. These control loci are used to estimate the absolute number of nucleated human cells in a biological sample and provide information on the quality of the reaction by estimating the number of molecules of template present in the PCR. This information aids in differentiating between lack of DNA and lack of T-cell derived DNA. If necessary, depending on the application, these control molecules can also provide a measurement of the T-cell fraction within a biological sample.

4. **PCR Amplification Controls:** A positive and negative amplification control are PCR-amplified along with each set (up to 22 samples) of test samples that are processed together. The amplification controls are subsequently prepared for sequencing and sequenced in the same manner as the test samples. The amplification positive control consists of gDNA derived from peripheral blood mononuclear cells and serves as an additional check to confirm successful product amplification. The amplification negative control consists of DNA Suspension Buffer and allows identification of reagent contamination. The core data generated from these samples must pass all pass/fail data QC specifications.

5. **Sequencing Process Controls:** The Illumina PhiX sequencing control is added to every flow cell along with test samples (up to 48 samples) and is used to estimate sequencing error rate for every sequencing run. These controls ensure adequate amplification and assessment of coverage, accurate amplification bias correction, accurate quantification of TCR β sequences and acceptable error tolerance.

6. **Other Controls:** A previously characterized COVID-positive sample is included periodically (greater than once per week). The previously characterized positive sample serves as a positive control for the run. The control sample chosen for this purpose must have a positive score with sufficient certainty that it will return a positive score >95% of the time. However, it should also be sufficiently near the score threshold to indicate an assay run failure in the event of a problem. Therefore, the targeted COVID-score range for the sample is between 9.47 and 30, but others may be used if availability is limited. In the event of a failure, the cause of the failure is evaluated. Control tracking and trending will occur and be reviewed at least monthly.

INTERPRETATON OF RESULTS

All controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Table 3. Expected Results for the T-Detect COVID Test External Controls

External Control Type	Internal controls	Expected output for Pass	Action if Failed
Positive Extraction Control	N/A – Control is not amplified or sequenced	Quantification: > 40 ng/ul	CLD Review
Negative Extraction Control	Processed with same internal controls as clinical samples	Quantification: < 10 ng/ul	CLD Review
Positive Amplification Controls	Processed with same internal controls as clinical samples	Sample Level QC Flags	CLD Review
Negative Amplification Controls	Processed with same internal controls as clinical samples	Amplification: < 400 Internal Control Templates	CLD Review

Assessment of clinical specimens should be performed after the extraction and amplification controls have been examined and the well, sample and flow cell level QC assessment are determined to be valid and acceptable. Well level QC assesses the quality of the overall sequencing quality of the PCR products by quantifying the amount of sequence originating from both TCRs and synthetic control. Sample level QC assesses the agreement of the 4 replicate of each sample and flags samples with too many well level failures (>1). Flow cell level QC is used to assess the overall quality of the sequencing run by checking control measures such as the PhiX sequences and sequencing error rate. If the controls or QC assessments are not valid, the patient results cannot be interpreted. The score produced by the COVID-specific algorithm (classifier) will be used to determine patient infected status.

The T-Detect COVID Test Controls uses a score produced from the COVID-specific algorithm to determine the test result for a given sample. The classifier identifies and quantifies any SARS-CoV-2-associated TCRs from a predetermined list of several thousand SARS-CoV-2 associated TCRs and also quantifies non-SARS-CoV-2 TCRs. These two variables are used in their 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 interpretation and reporting of clinical specimens are summarized in Table 4.

Table 4. Result Interpretation for Patient Samples

Score	QC results	Controls results	Thresholds on number of unique TCR sequences	Clinical Result	Action
≥ 2.23	Pass	Pass	≥ lower positive threshold and ≤ upper threshold	Positive	Positive report
			> upper threshold	Positive	Retest with lower input
			< lower positive threshold	Positive	No Result report
		Fail	≥ lower positive threshold	Positive	CLD review
			< lower positive threshold	Positive	No Result report
	Fail	Pass or Fail	Not determined	No result	Retest per flowchart
< 2.23	Pass	Pass	≥ lower negative threshold and ≤ upper threshold	Negative	Negative report
			> upper threshold	Negative	Retest with lower input
			< lower negative threshold	Negative	No Result report
		Fail	≥ lower negative threshold	Negative	CLD review
			< lower negative threshold	Negative	No Result report
	Fail	Pass or Fail	Not determined	No result	Retest per flowchart

PERFORMANCE EVALUATION

1) Analytical Specificity:

Reactivity/inclusivity

The T-Detect COVID Test detects the T-cell immune response to SARS-CoV-2 infection. Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no strains soliciting a unique T-cell immune response have been described relative to the originally isolated virus (this research is exceptionally limited at present).

Analytical Specificity:

Analytical specificity was verified in a set of blood and PBMC samples collected from individuals infected with Influenza A/B, Haemophilus Influenzae b, HIV, HBV and/or HCV to assess potential cross-reactivity. The infection status of each individual is determined by the vendor using anti-body tests. No samples tested positive using the T-Detect COVID Test. The data is summarized in the following table:

Table 5. Cross Reactivity Study Result

Infectious Target	Number of Samples	Source/Type	Positive With T-Detect COVID Test
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

2) Clinical Evaluation:

Clinical Sensitivity

Clinical sensitivity was evaluated by conducting a primary study testing a total of 208 frozen whole blood specimens and a secondary study testing 51 frozen whole blood specimens. Samples in the primary study were collected from patients that tested positive with an FDA authorized molecular comparator test from a single site in New York. Samples in the secondary study were collected from patients across 24 locations in the U.S. and the patients were tested with a variety of FDA authorized reference methods performed by a number of different labs.

All specimens included in the clinical sensitivity study were tested using the T-Detect COVID Test at Adaptive Biotechnologies Corporation. Results of the secondary clinical study by days post symptom onset are presented in Table 6.

Table 6. Results of the secondary clinical study with samples collected from subjects confirmed to be SARS-CoV-2 positive by an FDA-authorized PCR by days post symptom onset.

Days Post Symptom Onset	Number Tested	Positive With T-Detect COVID Test	PPA (95% CI)
0 - 7	11	5	45.5% (16.7% - 76.6%)

8 - 14	2	2	100% (15.8% - 100%)
>= 15	38	35	92.11% (78.62% - 98.34%)
Total	51	42	

Since samples collected from the primary clinical study did not include days post symptom onset information, these samples were stratified by days since positive PCR. Positive Percent Agreement (PPA) and 95% confidence intervals are shown in Table 7. There were 3 of the 208 samples tested in the primary clinical study that did not return any result due to QC failure.

Table 7. Results of the primary clinical study with samples collected from subjects confirmed to be SARS-CoV-2 positive by an FDA-authorized PCR by days since positive PCR.

Days since Positive PCR	Number Tested	Positive With T-Detect COVID Test	PPA (95% CI)
0 - 7	35	25	71.4% (53.7% - 85.4%)
8 - 14	33	31	93.9% (79.8% - 99.3%)
>= 15	137	133	97.1% (92.7% - 99.2%)
Total	205	189	

Clinical Specificity

Clinical specificity was evaluated by conducting a retrospective study testing a total of 22 frozen whole blood specimens and a prospective study testing 79 frozen whole blood specimens. Frozen whole blood samples used in the retrospective study were collected from Oct. 2019 to Nov. 2019 from multiple U.S. location and are presumed to be SARS-Cov-2 negative. Frozen whole blood samples in the prospective study were collected from a single site in New Jersey. These prospective samples were collected from patients presenting with SARS-CoV-2 symptoms but testing negative for SARS-CoV-2 using an FDA authorized test.

Results of the prospective and retrospective clinical studies are presented in Table 8. There were 2 of the 22 samples tested in the retrospective study that did not return results due to QC failure.

Table 8. Results of the prospective and retrospective clinical study with samples collected from subjects confirmed to be SARS-CoV-2 positive by an FDA authorized PCR by day from symptom onset

Study	Number Tested	Negative With T-Detect COVID Test	NPA compared with expected result (95% CI)

Retrospective	20	20	100% (83.2% - 100%)
Prospective	79	78	98.7% (93.1% – 99.97%)
Total	99	98	

WARNINGS AND PRECAUTIONS:

• This test has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA.

• Testing of venous whole blood using K2 EDTA specimens is limited to laboratories designated by Adaptive Biotechnologies Corporation that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests as described in the T-Detect COVID Test Standard Operating Procedure that was reviewed by the FDA under this EUA.

• This test has been authorized only for detecting and identifying the presence of an adaptive T-cell immune response to SARS-CoV-2, not for any other viruses or pathogens.

• The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

• It is recommended that all specimens be handled in accordance with Biosafety Level 2 practices as described in the CDC/NIH Publication, Biosafety in Microbiological and Biomedical Laboratories or other equivalent guidelines.

• Always wear gloves when performing this procedure and treat all specimens and used devices as potentially infectious.

LIMITATIONS

1. Samples should only be tested from individuals that are 15 days or more post-symptom onset.
2. Use of the T-Detect COVID Test is limited to personnel who have been trained.
3. Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.

4. It is unknown at this time if the presence of a T-cell immune response confers immunity to re-infection.

5. Results from T-cell immune response testing should not be used to diagnose or exclude acute COVID-19 infection or to inform infection status.

6. False positive results may occur due to cross-reactivity or other possible causes.

7. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for additional testing.

8. Pedigreed specimens with direct evidence of previous non-SARS-CoV-2 coronavirus (common cold) strains such as HKU1, NL63, OC43, or 229E, have not been evaluated with this assay.

9. A negative result for an individual subject indicates absence of detectable T-cell immune response. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.

10. A negative result can occur if the T-cell immune response present in the specimen is below the detection limits of the assay, or the T-cell immune response is not present during the stage of disease in which a sample is collected.

11. Not for screening of donated blood.

12. The performance of this test has not been established in immunocompromised individuals.

13. The performance of this test has not been established in individuals that have received a COVID-19 vaccine. The clinical significance of a positive or negative result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.

14. The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.