Escher BT-MED® COV19 Test (Saliva)

Qualitative Test for SARS-CoV-2
Used with the Escher BT-MED® Analyzer

Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2

Validation of this test has not been reviewed by FDA.
Review under the EUA program is pending.

Escher BT-MED® COV19 Test Kit, and **Escher BT-MED® Analyzer**

Intended Use:

Escher BT-MED® COV19 Test is a nucleic acid molecular probe as detected by automated epifluorescence microscopy intended for the detection of RNA from SARS-CoV-2 in fresh or preserved saliva collected in sterile specimen containers from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in saliva specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Escher BT-MED® COV19 Test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in in vitro diagnostic procedures and in the use of the Escher BT-MED® Analyzer and Escher BT-MED® COV19 Test Kit.

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DEVICE DESCRIPTION AND TEST PRINCIPLE

Summary of the Product and Test Principle:

The **Escher BT-MED® COV19 Test Kit** and the **Escher BT-MED® Analyzer** operate together to determine the presence or absence of SARS-CoV-2 virus in a fresh or preserved saliva specimen.

The **Escher BT-MED® COV19 Test Kit** is a set of consumable reagents and items necessary for the test to be performed. The Test Kit includes reagents to perform the test, as well as positive and negative control materials. The Test Kit also includes the test disks that hold 18 sample cups held at its periphery. The Test Kit contains the components for 540 (Test Kit version A) or 1080 (Test Kit version B) assays.

The Test is a machine-based molecular-probe fluorescence-in-situ-hybridization method, with automated processing and read-out. The assay detects and quantifies SARS-CoV-2 / COVID-19 virus by means of the in-situ-hybridization of viral RNA in host cells during or after viral replication, using a fluorescently labeled single-strand DNA-probe specific for the nucleocapsid-gene of the SARS-CoV-2-virus.

The test is qualitative, and evaluation of the presence or absences of the SARS-CoV-2 virus occurs automatically within the system, returning the result to the laboratory user as YES, NO, HELP/REPEAT as to the presence of the virus.

The **Escher BT-MED® COV19 Test Kit** is to be used together with the **Escher BT-MED® Analyzer**.

The **Escher BT-MED® Analyzer** is a flexible automated benchtop platform for the preparation and analysis of biomedical samples with a focus on pathogens. For this high throughput SARS-CoV-2 detection method, the analyzer-automated preparation functions are suppressed or not present.

In use with the COV19 Test Kit, the Escher BT-MED® Analyzer system includes

1) Electronic user interface.
2) Sample analysis chamber fitted for the capture of epifluorescence micrographs of cellular samples from within the sample cups.
3) Image capture system and digital-signal-processor.
4) Artificial intelligence/neural network (AI/NN) process program.
5) Results-reporting user interface.
6) Programmable electronic laboratory or hospital reporting interface.

A full or partial disk of 18 sample cups is loaded into the analyzer, having sample ID numbers associated with the respective samples. Processing is automatic and results for a full disc holding 18 sample filters become available in 30 minutes.

The test is qualitative, and test evaluation is made automatically within the system, returning the qualitative result to the laboratory user.

Specimen collection occurs by the test subject depositing saliva directly into a standard sterile medical specimen container.

Specimen handling and holding: The saliva specimen may be transported to the clinical laboratory, and prior to testing may be held at 4°C or 25°C. See the section below on Specimen Stability.

Specimen Preservation can be achieved by mixing of the saliva with an Escher-proprietary ethanol-based preservation fluid in a 1:1 volume ratio. This offers the advantages of longer possible holding times at 25°C, sterilization of the saliva specimen and a reduction in the in-laboratory steps necessary to process the specimen.

Pre-analytic steps of specimen processing occur on the lab bench by a trained technician, and include fixing, COV19 Test Kit-specific molecular-probe staining and de-staining steps, centrifugation and sub-sampling onto a 0.12" (3 mm) 'filter' that is specific to the Escher BT-MED® COV19 Test Kit for use in the Analyzer.

Equipment necessary for these pre-analytic steps include personal protection equipment, pipettors, centrifuge, drying ovens, and appropriate waste disposal.

Analysis is performed automatically after the 9" (22 cm) analysis disc holding 18 'filter cups' is loaded into the Escher BT-MED Analyzer. The automated analysis is based on the combination of epifluorescence microscopy, three-dimensional imaging, digital image capture, followed by neural network-assisted evaluation of the samples on each filter. This evaluation includes assessment as to whether the morphology of the objects in the sample images is in accordance with pre-existing rules applying to the COV19 with respect to human cells and viral molecules therein.

The test result is interpreted by the Analyzer system software using specific COV19 analysis rules and clinically-validated interpretation, and converted to result messages per sample:

- YES, (virus detected)
- NO, (virus not detected)
- HELP/REPEAT (sample not able to be analyzed)

TEST STEPS:

Please see *Escher BT-MED® COV19 Saliva Protocol* appearing at the end of this *Instructions for Use* document.

INTERPRETATION OF RESULTS:

Assessment of clinical specimen test results should be performed after the system, positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

See the below table in the section on Controls to ensure that only valid results are considered.

The COV19 Test Kit assay results are presented as:

YES	(virus is present),
NO	(virus is not detected)
HELP/REPEAT	(the sample cannot be analyzed)

No interpretation, curve review, or post-analytical calculation is necessary.

CONTROLS:

Control Material(s) to be used with Escher BT-MED® Analyzer and BT-MED® COV19 Test Kit include:

a) A negative control sample ("NEG"), is included with the Test Kit. When 10ul of this material is tested according to its instructions, and using the Negative Control (POS/NEG Check) setting on the Escher BT-MED® Analyzer, an assay is performed to demonstrate that the Analyzer is operating correctly. The negative control sample is molecular grade RNAase-free water that does not contain any targets. System results indicate if the result passes this negative control test.

b) A positive control sample ("POS"), is included with the Test Kit. When 10ul of this material is tested according to its instructions, and using the Positive Control (POS/NEG Check) setting on the Escher BT-MED analyzer, an assay is performed to demonstrate that the BT-MED is operating correctly. The positive control sample is a virus-free mixture that contains specific microscopic fluorescent objects which act as proxies for infected cells, and will indicate a positive result. System results indicate if the result passes this positive control test.

These control materials validate that the Escher BT-MED® Analyzer is operating correctly in evaluating the absence or presence of objects of correct morphology and fluorescence properties, and that the laboratory operator has sampled and placed the control materials correctly into the test disk/cups.

At the laboratory's discretion, additional validation may be performed using specimens obtained from patients of known SARS-CoV-2 infection status.

Internal controls within the **BT-MED® Analyzer,** requiring no additional action by the laboratory user, include:

c) System self-tests and internal tests occur at startup time, and continue during idle time and operation time to evaluate and monitor correct values of system variables.

d) Run-time self-testing, internal tests and sample characteristics are monitored during the automated observation and pre-processing stages of the system process.

e) Internal observations and computational steps make evaluations of suitability of the measurements made on the sample to allow a YES/NO determination of test results to be made. This suitability test includes evaluations that can exclude specimens that are incorrectly prepared during the pre-analysis steps, including poor performance of sample collection, lysis, isolation, probe staining, washing and sample application steps. Unsuitable samples result in a HELP/REPEAT message. This set of tests acts as an internal control for each sample.

Interpretation of patient test results is possible only when System Self-Tests perform normally, that is, without reporting an error as "ERROR". In the event of this failure the system locks out the possibility of conducting tests.

In addition, the POS and NEG check tests must have passed as reported by the "POS=POS" and "NEG=NEG" messages. In the event that the System Self-Tests are normal, and the POS/NEG checks are passing, then the test result may be considered valid (see Interpretation of Results, above).

Additional information regarding the next appropriate actions in the event of these messages (ERROR, FAIL, HELP/REPEAT) are included in the TROUBLESHOOTING section below and in the BT-MED Analyzer *User Manual*.

Positive and Negative control tests (POS, NEG) should be performed according to the laboratory's program of quality control, and can be performed upon the arrival of a new lot of Escher BT-MED COV19 Test Kits, once per week, once per day or once per shift.

Performance of Positive and Negative control tests may be included in two of the 18 sample cup/position/filters immediately prior to routine testing. These control positions must be correctly identified to the Escher BT-MED Analyzer prior to starting the control run.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, any subsequent patient test results cannot be interpreted.

See the table in the section below showing status of the control tests that provide valid and invalid results, respectively.

Escher BT-MED®COV19 Controls, Results and Interpretation:

System Self-Tests	**POS test**	**NEG test**	**Test Result**	**Status**
(Normal)	**PASS (POS=POS)**	**PASS (NEG=NEG)**	**YES**	**Test Result Is Valid Virus is Present**
(Normal)	**PASS (POS=POS)**	**PASS (NEG=NEG)**	**NO**	**Test Result Is Valid Virus is Not Detected**
(Normal)	PASS	PASS	HELP/REPEAT	**Test Is Invalid** (at that cup position)
(Normal)	**FAIL**		YES/NO	Tests are invalid
(Normal)		**FAIL**	YES/NO	Tests are invalid
(Normal)	**FAIL**	**FAIL**	YES/NO	Tests are invalid
ERROR	-	-	-	System allows no tests/lock out

COMPONENTS INCLUDED WITH THE TEST KIT

Components supplied with the Escher BT-MED® COV19 Test Kit:

(Test Kit contains 30 Analysis Disks, 18 filter cups each disk, for 540 Analyses total)

Item	Description	Quantity	Part Number
1	SARS-CoV-2 (COV19) probe.	30x1 ml	Biotrack FP02400
2	Negative control assay.	1x 1 ml	Biotrack FP02401
3	Positive control assay.	1x 1 ml	Biotrack FP02402
4	Washing buffer (WSHB)	1x 1000 ml	Biotrack FP03150
5	Analysis Disk with 18 filter cups	30x filter disks	Biotrack FP01002
6	Control Disk with 18 filter cups	1x filter disk	Biotrack FP01200
7	Saliva Fixative Kit	1 liter	Biotrack FP02480

Components Required But Not Included with the Test

Consumables-Description

Sterile and re-sealable specimen collection containers, 25-50 ml (See specification below†)
Water, deionized, Milli-Q (ultrapure water type I)
Sterile isotonic salt solution (0.9% (m/v))
Methanol (>99% v/v) **OR** Escher BT-MED® Saliva Fixative Kit**
Eppendorf tubes 1.5 ml
Sterile filter pipette tips 100-1000µl
Sterile filter pipette tips 2-200µl
Waste container (biological liquid waste)
Waste container (biological solid waste)
*Escher Biotrack COV19 probe (stored at 4°C (39°F), dark conditions)
*Escher Biotrack Wash-buffer (WSHB) (preheated 60°C (140°F))
*Escher BT-MED analysis disk (18 cups)

* (COV19 Test Kit Cat. No FP02400) contains these components
** (COV19 Saliva Fixative Kit Cat. No FP02480) contains this component

Equipment-Description

Escher Biotrack BT-MED® Analyzer
Laminar flow cabinet
Incubator 50°C (122°F), 60°C (140°F)
Eppendorf centrifuge with setting for 800 X g with soft acceleration and deceleration
1000µl pipette, several recommended
100µl pipette, several recommended
Timer / Stopwatch (adjustable)
Test tube rack for medical specimen collection containers
Eppendorf tube rack, multiple recommended

† Specimen collection containers can be obtained either pre-sterilized or non-sterilized. In the case of non-sterilized containers, standard autoclave procedures may be used for sterilization.

Specimen collection containers must meet these requirements:

- Fabricated of polypropylene or similar transparent (or translucent) material.
- Sterile, or suited to be sterilized, such as by autoclave.
- Sterile at the time of specimen collection.
- Of 25 to 50 ml total volume.
- Opening of approximately 0.75” to 1.5” (~2-4 cm) to facilitate saliva collection.
- Graduations/volume indications on the container side.
- Liquid-tight re-sealable screw cap.

Suppliers of suitable 50 ml culture/centrifuge tubes and/or 25-50 ml specimen collection containers used for the Clinical Evaluation included:

- VWR / Avantor
- Thermo-Fisher Scientific
- Sigma Aldrich/Merck
- Greiner Bio-One

STABILITY

Stability studies were performed according to the CLSI EP25-A standard.

Reagent Shelf-Life:

The Escher BT-MED COV19 molecular probe and wash buffer reagents is demonstrated to be stable to 6 months with real-time aging, per a studies performed according to the CLSI EP25-A standard.

See the packaging for the Escher BT-MED® COV19 Test Kit for shelf-life expiration of the liquid reagents. Do not attempt to use reagents that are past the expiration date.

Reagent Open-Item Stability:

Test Kit reagents must be kept with the containers closed when not withdrawing material for immediate use. Reagent items that are left at room temperature and open must be used prior to 90 minutes. Re-closed and refrigerated reagent items can be used as long as to 120 minutes.

Saliva Specimen Stability:

Saliva specimen stability, in dark conditions, was found to be:

Fresh Saliva	25°C	21 hours
Fresh Saliva	4°C	42 hours
Preserved Saliva	25°C	144 hours

Warnings and Precautions

- For in vitro diagnostic use only.
- For prescription use only.
- Validation of this test has not been reviewed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2.
- This test is for use by laboratories certified under CLIA, 42 U.S.C 263a, that meet the requirements to perform high complexity tests.
- Use Escher BT-MED® COV19 Test Kit with the Escher Biotrack BT-MED® Analyzer only. Do not use test kit if there is evidence of damage or leakage.
- Avoid contact with eyes and skin. Flush with water if the solution is spilled or in contact with skin.
- Use standard biohazardous material safety precautions when collecting and handling samples.
- Used materials are potentially infectious and should be discarded in accordance with local, state and federal regulations.

PERFORMANCE EVALUATION

Limit of Detection (LoD) - Analytical Sensitivity:

This study follows the CLSI EP17-A2 guideline document in the implementation of this study, including in particular the Probit Approach outlined in section 5.5 of that document.

The study was conducted with specimens of clinically-acquired saliva specimens from patients collected in specimen containers as described elsewhere in this document, in this case, sterile 50 ml culture tubes.

Per guidance, a preliminary LoD was estimated through a study in which 100% of observations were positive at a tested concentration of 1.00 copies per microliter.

In this final LoD study, three independent source positive control saliva specimens were created by a certified external laboratory, using quantified known positive clinical specimens. Samples positive for virus were made with saliva specimens from patients with positive SARS-CoV-2 RT-PCR results, and with symptoms of COVID-19, as well as subsequent positive serology ELISA for IgG/IgM. The concentration series of the respective specimens was prepared after confirmation of the concentrations of virus/analyte using the (FDA EUA Authorized) Roche cobas® SARS-CoV-2 test, performed on the cobas® 6800 RT-PCR system with cobas® test kits. Zero level virus/analyte specimens were made with saliva from patients with negative RT-PCR SARS-CoV-2 results. Dilution of three independent positive specimens was made with appropriate uninfected saliva specimens to create three independent sets of the non-zero concentrations of virus/analyte.

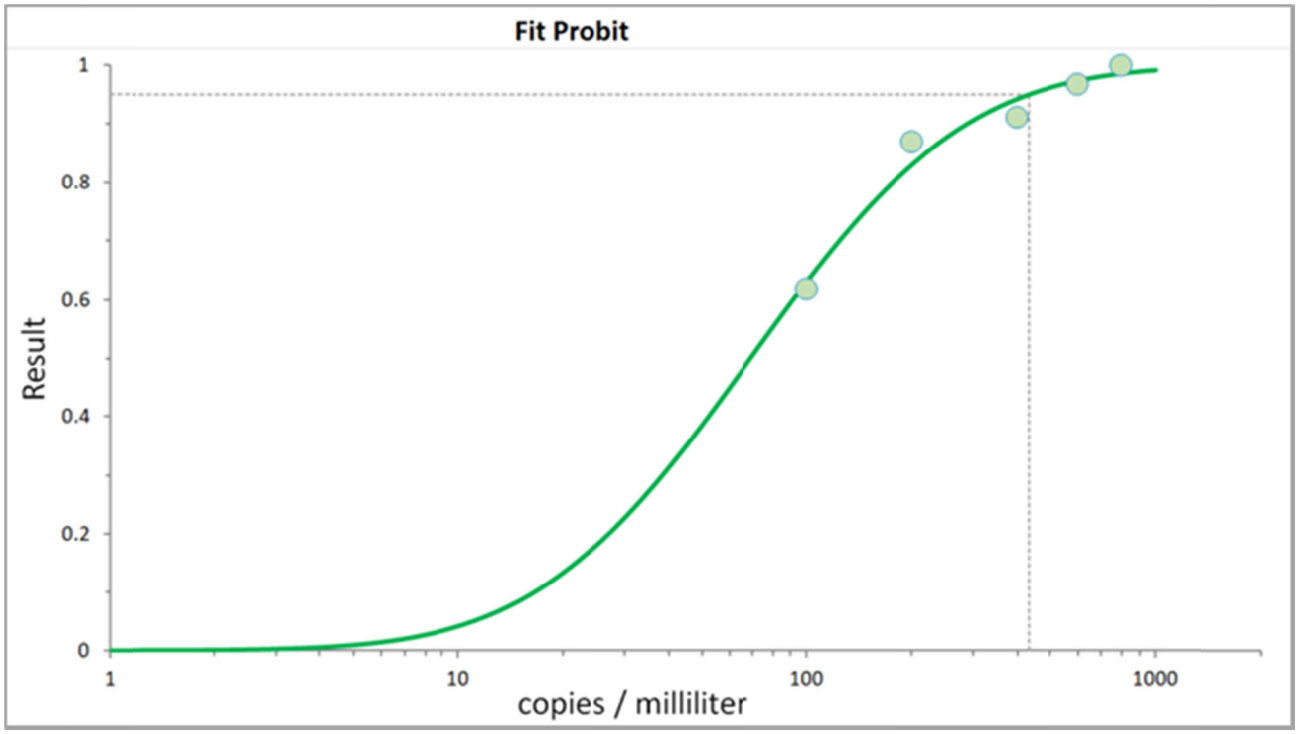
The five non-zero concentrations tested were 0.80, 0.60, 0.40, 0.20, 0.10 copies per microliter. The LoD study design used 3 independently acquired specimens, 5 non-zero concentrations, 3 days of testing, 2 lots of BT-MED® COV19 Test Kit materials, and 20 replicates per condition. This resulted in 1800 total observations with 360 observations at each of the 5 non-zero concentrations.

Probit analysis was performed per guidance using Analyse-It® for Microsoft Excel 5.68 (Ultimate Edition). Estimates of LoD (95%) were obtained using fits of the lowest 3 concentrations, the lowest 4 concentrations and all 5 concentrations:

Probit Fit Data Points	LoD (95%), Concentration, copies per microliter
All 5 concentrations	0.43 copies / microliter
Lowest 4 concentrations	0.48 copies / microliter
Lowest 3 concentrations	0.49 copies / microliter

The Level of Detection (95%) was found to be 0.49 copies per microliter.

This value was confirmed by the testing of 360 replicates at 0.80 copies per microliter (80% of the concentration of the preliminary LoD) with each replicate proceeding through the pre-analytical and analytical stages of the assay independently, in which 100% (360/360) of the results returned as positive.


Fit Probit
Result
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
copies / milliliter

Inclusivity (analytical sensitivity):

The molecular probe in the COV19 Test kit is specific to a proprietary (~30 bp) invariant sequence of the nucleocapsid of SARS-CoV-2 coronavirus. 100% of the published sequences of the virus genome nucleocapsid sequences are detectable with the COV19 molecular probe (as of 1/11/2021).

Cross-reactivity (analytical specificity):

Recall that the Escher BT-MED COV19 Test Kit assay utilizes specimens of saliva as the sample matrix, and that the assay analyte is viral particle components, specifically N-gene mRNA, enclosed within human cells from the specimen.

Analytical Specificity/Cross-reactivity of the Escher BT-MED COV19 Test Kit was evaluated using both *in silico* analysis and by wet testing with whole organisms listed in the table below. The empirical testing showed that the results were negative for all tested microorganisms except for the SARS-CoV-2 virus which is expected to react with the probe (target for the universal detection of SARS-like viruses) which is part of the Escher BT-MED COV19 test.

The COV19 molecular probe is not seen to cross-react with SARS-CoV-1 and MERS-CoV; in the clinical risk of such cross-reactivity is unlikely due to very low prevalence of these viral pathogens, and therefore clinically unimportant for the intended use.

Microbial Interference Studies:

Empirical wet testing was performed by using Escher BT-MED® COV19 Test Kit and Escher BT-MED® Analyzer using the appropriate controls on a series of test organisms. These organisms were procured from DSMZ Culture Collection (Braunschweig, Germany). All organism preparations were of concentrations between 1E+05 and 1E+06 $TCID_{50}$ / milliliter. The results indicated that no significant positive signal could be acquired from the organisms tested, as expected and shown in the below:

Cross-Hybridization Test Results—Wet Testing	
Organism	***Escher BT-MED COV19 SARS-CoV-2 result***
Adenovirus 11	NO / Not detected
Adenovirus 5	NO / Not detected
Bordetella pertusis	NO / Not detected
Chlamydia pneumoniae	NO / Not detected
Enterovirus 70	NO / Not detected
Haemophilus influenzae	NO / Not detected
Human metapneumovirus	NO / Not detected
Human para influenza virus 1	NO / Not detected
Human para influenza virus 2	NO / Not detected
Human para influenza virus 3	NO / Not detected
Human para influenza virus 4b	NO / Not detected
Human respiratory syncytial virus	NO / Not detected
Influenza Virus A	NO / Not detected
Influenza Virus B	NO / Not detected
Legionella pneumophila	NO / Not detected
Mycobacterium tuberculosis	NO / Not detected
Mycoplasma pneumoniae	NO / Not detected
Streptococcus pneumoniae	NO / Not detected
Streptococcus pyogenes	NO / Not detected

***In silico* analysis** was performed as well, showing no homology of the COV19 fluorescent molecular probe for the genomes of the organisms listed in the table below:

In silico analysis did not reveal any homology ≥ 80% with microorganisms of interest.

In silico analysis of the DNA-probe contained in the Escher BT-MED COV-19 Test Kit	
Probe Parameter	***Score***
GC%	**65%**
Tm	**51 Celsius**
Hairpin(s)	no
Internal duplex formation	no
Best mismatch	83%
Homology Adenovirus 11	0%
Homology Adenovirus 5	0%
Homology Adenovirus 11	0%
Homology parainfluenza 1	0%
Homology Parainfluenza 2	0%
Homology Parainfluenza 3	0%
Homology Influenza a	0%
Homology Influenza b	0%

Additional Interferent Discussion:

Validation included interference testing appropriate for the BT-MED® COV19 Test for use with fresh saliva specimens. (See the section above on Microbial Interference Studies including *in vitro* and *in silico* testing).

The foundation of the BT-MED® COV19 Test is the utilization of 1) fluorescent *in situ* hybridization with nucleic-acid-sequence-specific molecular probe, and automatic artificial intelligence-driven evaluation of the data sets that consider only the 2) intracellular volumes of human cells, only 3) moderate to large copy numbers of viral gene targets, and only those cells that exhibit the distribution of hybridized molecular probe in a pre-determined morphology 4) consistent with SARS-CoV-2-infected cells. The union of these four requirements form the criteria by which the presence or absence of the virus is determined.

The category of potential interferent that could present as a false-positive result for this test would be an (human cell) intracellular source of SARS-CoV-2 nucleocapsid RNA that becomes replicated to at least moderate copy number, and becomes distributed into a morphology consistent with the human cell infected with the virus. Presently there is no known non-SARS-CoV-2 microorganism or material capable of meeting the four key requirements in order to cause a false-positive result.

Other potential interferents that might increase the background fluorescence of the specimen cannot cause false-negative results because if the background intensity reaches a sufficient level to interfere with the assay, the above requirements cannot be evalulated, and the analysis algorithm declares ‘Can Not Analyse’, prevents a Yes/No result from being generated, and instead returns “HELP/REPEAT” as a null test result.

Key BT-MED® COV19 Detection Criterion The candidate test material must be:	**Why the Criterion is Appropriate to the BT-MED® COV19 Test with saliva specimens:** In testing as to the presence or absences of the SARS-CoV-2 virus:
A. <u>Hybridize with Molecular Probe:</u> Capable of highly specific hybridization with the nucleic-acid-sequence-specific molecular probe that is shown to hybridize only with SARS-CoV-2.	The assay evaluates only COV19 molecular probe that is hybridized to a highly conserved portion of the SARS-CoV-2 virus nucleocapsid gene.
B. <u>Intracellular:</u> Capable of entering and existing in the intracellular space of the human cells from matrix that are being tested for the presence of the virus.	The assay evaluates only materials within intact human cells (at the time that the specimen was mixed with fixative/preservative). The automated analysis of the fluorescent *in situ* hybridization images excludes any portion of the specimen that is not within the intracellular space of human cells.
C. <u>Moderate to High Copy Number:</u> Capable of existing in the intracellular space in sufficient copy number to become detectable by the method.	The assay evaluates only portions of the specimen with a significant number of virus/analyte molecules within a cell. Infection by SARS-CoV-2 can involve multiple infecting virions per cell, as well as replicated virions, but also may exhibit multiple mRNA replicates of the nucleocapsid gene, sometimes 10^2 to 10^3 relative to genomic RNA.
D. <u>Matches Specific Pre-Determined Morphology of Infected Cells:</u> Capable of existing in intracellular space in the spatial distribution, concentration and intensity that meets the pre-determined definition for the morphology of a virus infected cell, in this case, that of SARS-CoV-2.	The assay evaluates only those portions of the specimen that match the morphology of a human cell infected by the SARS-CoV-2 virus. Images of human cells infected by the virus exhibit a characteristic appearance of molecular probe in one to many centers distributed within the cell. The automated BT-MED® Analyzer applies pre-determined criteria regarding dozens of morphological metrics in order to establish a match as an infected cell.

Material/Item	Meets All 4 Key BT-MED® COV19 Detection Criteria (A, B, C, D, to the right)	A. Hybridizes to Molecular Probe	B.Intracellular	C. Moderate or High Copy Number	D. Correct Specific Intracellular Morphology
Known Positive Assay Materials:					
SARS-CoV-2 infected human cells	YES (Established and Validated)	Yes	Yes	Yes	Yes
Infected Blood[1] (cells)	YES (Established)	Yes	Yes	Yes	Yes
Potential Interferents:					
Non-SARS-CoV-2 Microorganisms[2]	NO	No	Yes	Yes	Maybe
Mucin from saliva	NO	No	No	No	No
Uninfected Blood[1] (cells or plasma)	NO	No	No	No	No
Toothpaste	NO	No	No	No	No
Mouthwash	NO	No	No	No	No
Tobacco, residual	NO	No	Maybe	No	No
Food, residual	NO	No	No	No	No
Cosmetics	NO	No	Maybe[4]	No	No
SARS-CoV-2 Vaccine, nano-particle with viral genetic material[3]	NO	No[3]	Yes	Maybe	Unknown

[1] Blood is generally removed from the saliva sample during the first rinse step of specimen collection. Large quantities of blood may cause the assay to fail, but not return an erroneous result. Any material that disables the ability of the assay to identify properly formed human cells returns a HELP/REPEAT message to the user, indicating that the system cannot make a determination as to the presence or absence of the virus.

[2] Please refer to the section regarding Microbial Interference Studies. None of the microorganisms studied cross-react with the COV19 molecular probe.

[3] Vaccine-related nanoparticles carrying SARS-CoV-2 viral RNA sequences exist. However as of 1/2021, no N-gene-based vaccines are reported, even in development. It is not clear that material from a vaccine agent will be present in blood or saliva cells in any appreciable concentration, and it is unknown as to the copy number levels or morphology of intracellular localization nucleic acids due to such a vaccine if it existed.

[4] Some cosmetics contain nanoparticle objects that can theoretically arrive in the intracellular space of human cells.

CLINICAL EVALUATION

A clinical study was performed with 491 subjects, including those with symptoms of COVID-19 or other respiratory illnesses, those with suspected contact with COVID-19 individuals, and those without either.

491 paired natural clinical specimens were obtained as nasopharyngeal swab for the reference method, and as saliva specimens for the Escher BT-MED® COV19 Test. Paired specimens were collected simultaneously, within no more than 24 hrs between collections.

Of these specimens:

- 361 were from subjects that were tested and SARS-CoV-2 infection was not seen.*
- 130 were from subjects that were confirmed to have SARS-CoV-2 infection*.
    - Of these 130, the number having RT-PCR C_t values ≥ 30 and considered 'low-positive' was 80 specimens/subjects.

* Clinical status of subjects was made by use of RT-PCR testing with the EUA-Authorized Roche cobas® 6800 instrument and associated Roche SAR-CoV-2 cobas® test kits as the reference method.

Escher BT-MED® COV19 Test (Saliva) vs. RT-PCR Swab Reference Standard

Frequencies

		Escher BT-MED® COV19 Test		
		NEG	POS	Total
RT- PCR Swab	NEG	356	5	361
	POS	4	126	130
	Total	360	131	491

Proportions

	Agreement	95% CI (Wilson)
NPA	0.986	0.968 to 0.994
PPA	0.969	0.924 to 0.988

LIMITATIONS:

Validation of this test has not been reviewed by FDA. Review under the EUA program is pending. Distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency, Section IV.C.2

Test reports to healthcare providers should include a statement to that effect, such as **"This test has been validated but FDA's independent review of this validation is pending."**

Escher BT-MED® COV-19 Test Kit – SALIVA Protocol

1. INTRODUCTION

This protocol describes the procedure for the fixation and analysis of saliva samples to detect SARS-CoV-2 (COVID-19) using the Escher BT-MED® COV119 Test Kit, the Escher BT-MED® Analyzer and the optional Escher Saliva Fixative Kit. The fixation procedure should be performed as soon as possible after collection of the sample. The preserved/fixed saliva sample can be stored (cold, dark conditions) for longer times than fresh saliva and is safer to transport and process.

This protocol describes the actions taken on a single specimen. In actual laboratory operations, multiple specimens will be processed in parallel or near-parallel operations as 'batches'. In addition multiple batches can be processed with staggered start times to maximize throughput.

2. Work Areas

BSL-2 space/laminar flow hood is recommended for operations involving un-fixed specimens and samples. General clinical laboratory space may be used for operations when samples are contained in sealed, cleaned tubes, or when samples are chemically fixed and can be safely removed from the BSL-2 space.

3. PROCEDURE

3.1 Obtaining a saliva specimen

- Provide a sterile and re-sealable specimen collection tube to the subject being tested.
- The subject must rinse their mouth with water for about 10 seconds, and then wait 5 minutes.
- After 5 minutes, the subject must spit approximately 5 ml of saliva into the specimen collection tube and carefully reseal it.
- The surface of the specimen tube should be wiped with an alcohol wipe to reduce the risk of transfer of materials with the potential to carry virus. ☣

3.2 Storage, handling and preservation of the saliva specimen

- Prior to analysis the fresh untreated saliva sample can be stored for the following times under the conditions shown:
  - 21 hours at 25°C, absence of light.
  - 42 hours at 4°C, absence of light.
- Alternatively, the saliva specimen can be mixed thoroughly in a 1:1 ratio with a preservative/fixative fluid mixture provided in the Escher **Saliva Fixative Kit**. This proprietary Biotrack/Escher product is an ethanol-based blue fluid that serves to preserves the saliva specimen.
- Prior to analysis The fixed/preserved saliva sample can be stored for the following times under the conditions shown:
  - 144 hours at 25°C, absence of light.

3.3 Process preparation

- Verify the immediate availability of necessary materials and supplies.
- Prepare 100-1000 µl and 20-200 µl pipettes for use.
- Prepare the solid and liquid waste containers.
- Take the saliva sample and put it in a test tube rack. Label an empty 1.5 ml Eppendorf tube with the corresponding sample identifier and place in an Eppendorf tube rack.

3.4 Saliva sample fixation (in the laminar flow cabinet)

IMPORTANT: If the saliva specimen has been mixed with Escher Saliva Fixative in step 3.2 above, then omit this step 3.4 and continue with step 3.5

- Add 1:1 methanol by volume to the specimen collection tube containing the saliva sample.
- Mix well with a 1000 µl pipette.
- Incubate for at least 10 minutes at 25°C. After incubation, subsequent procedure steps can be started immediately, or the fixed sample can be stored at 25°C in dark conditions for as long as 6 days.

3.5 BT-MED® COV19 Molecular Probe analysis with saliva

Escher Biotrack FISH Saliva staining procedure:

- Add 100 µl of Escher BT-MED® COV-19 probe to the empty Eppendorf tube.
- Homogenize the fixed saliva sample by 10 repetitions of thorough in-and-out pipetting (shearing) with a 100-1000 µl pipette (1000 µl), followed by 5 repetitions of thorough in-and-out pipetting (shearing) with a 20-200 pipette (50 µl).
- Pipette directly 50 µl of the homogenized saliva sample in the Eppendorf tube with the Escher BT-MED® COV-19 probe and mix 5-10 times with in-and-out pipetting.
- Incubate the Eppendorf in the dark for 90 minutes at 50°C (122°F).
- After incubation, add 1 ml of warm 60°C (140°F) Escher Biotrack Wash-buffer (WSHB) to the Eppendorf tube and mix well with 5 repetitions of in-and-out pipetting with a 100-1000 µl pipette (1000 µl).
- Incubate the sample in the dark for 30 minutes at 60°C (140°F).
- Centrifuge at 800 x g for 10 minutes.
- Carefully aspirate the supernatant with a pipette, so that pellet remains in the Eppendorf tube.
- Pipette 50 µl ultrapure water/Milli-Q into the Eppendorf tube and resuspend the pellet by 10 repetitions of in-and-out pipetting.

Subsamping Spot procedure:

⚠ **IMPORTANT: The black filter at the bottom of the sample cup is delicate, do not touch the filter with the pipette tip.**

- Take a clean Escher Biotrack Analysis Disk and carefully apply a 30 µl droplet of properly mixed sample from the Eppendorf to the sample cup at the edge of the disk. Let the surface tension of the droplet pull the liquid sample droplet onto the black filter at the bottom of the cup; do not touch the pipette tip to the cup. Make note of the sample identity and the sample cup number.

- Place the disk in the dark at 50°C (122°F) to dry the spotted samples. This is expected to take 25 minutes (or longer until the fluid is totally dry).
- After drying, the filter disk is loaded into the Escher BT-MED® analyzer.
- In the Escher BT-MED® Analyzer software, enter the specimen identifier in the "sample" field corresponding to the filter cup position on the filter disk. Select "COV-19 Saliva" as the program/protocol.
- Once the entries have been made and verified, initiate the analysis by select the "START" button on the software interface.

⚠ **IMPORTANT: Directions for operating the Escher BT-MED analyzer appear in the Escher Biotrack BT-MED User's Manual.**

3.6 Finishing:

Clean the workplace. Close waste containers tightly and dispose of according to your laboratory's procedures regarding (biohazardous) waste. Once analysis is complete, remove the BT-MED® disk from the Analyzer and dispose of properly.

3.7 Warnings and Precautions

Standard precautions apply to the BSL-2 working environment. Please adhere to the policies and procedures of the laboratory and institution.

END

CE IVD **In Vitro Diagnostic device, for professional use only**

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REF

Biotrack-MED® **Analyzer** BTMED20.x.xx
Escher BT-MED© Analyzer BTMED20.x.xx

REF Biotrack/Escher Catalog Numbers:

COV19 **Test Kit** Cat. No FP02400 (A/B)
COV19 **Saliva Fixative Kit** Cat. No FP02480 (A/B)