21120.10120 SARS-CoV-2 Next Generation Sequencing Procedure

Copy of version 3.0 (approved and current)

Last Approval or Periodic Review Completed	02-Mar-2021
Next Periodic Review Needed On or Before	02-Mar-2022
Effective Date	09-Mar-2021

Controlled Copy ID 308478

Location client request

Organization Eurofins - Viracor

Comments for version 3.0
Removed (b) (4) from SOP because only an (b) (4) is plated for this assay.

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	02-Mar-2021 11:06	3.0	(b) (6) (b) (6)	
Approval	(b) (6) Approval	01-Mar-2021 21:03	3.0	(b) (6) (b) (6)	
Approval	(b) (6) Approval	01-Mar-2021 17:02	3.0	(b) (6) (b) (6)	
Approval	(b) (6)	18-Feb-2021 13:56	2.0	(b) (6) (b) (6)	
Approval	(b) (6)	25-Jan-2021 13:16	2.0	(b) (6) (b) (6)	

Approval	(b) (6)	24-Jan-2021 20:21	2.0	(b) (6) (b) (6)
Approval	(b) (6) Approval	22-Jan-2021 18:47	2.0	(b) (6) (b) (6)
Approval	(b) (6)	12-Jan-2021 9:17	1.0	(b) (6) (b) (6)
Approval	(b) (6) Approval	09-Jan-2021 20:41	1.0	(b) (6) (b) (6)
Approval	(b) (6) Approval	05-Jan-2021 9:31	1.0	(b) (6) (b) (6)

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved and Current	Major revision	01-Mar-2021	09-Mar-2021	Indefinite
2.0	Retired	Major revision	22-Jan-2021	25-Jan-2021	09-Mar-2021
1.0	Retired	Initial version	04-Jan-2021	12-Jan-2021	25-Jan-2021

BioPharma Procedure for the SARS-CoV-2 Next-Generation Sequencing Assay

Table of Contents

FDA-CBER-2022-1614-1790492

Confidential Materials. Property of Viracor Eurofins.

INTENDED USE

The intended use of the assay is to provide SARS-CoV-2 nucleotide sequence determination for the S Gene by Next-Generation Sequencing.

TEST INFORMATION

Assay name: SARS-CoV-2 NGS

Date test initially placed into service: 25-Jan-2021

METHOD PRINCIPLE

Next Generation Sequencing

This assay uses conventional PCR chemistry for amplification of a SARS-CoV-2 target sequence from nucleic acids that have been extracted from nasopharyngeal swab specimens. The amplification step is followed by purification of the PCR products which is followed by visualization and concentration of the PCR products using the Agilent 2200/4200 TapeStation. Amplified samples are diluted, and libraries are prepared and indexed using the Illumina Nextera XT Library Prep Kit. Following a bead-based purification step, prepared libraries are quality checked on the Agilent 2200/4300 TapeStation. Libraries are then diluted, pooled, denatured, and loaded into a sequencing reagent cartridge for processing on the Illumina MiSeq instrument.

SPECIMEN REQUIREMENTS

Patient Preparation:

- No preparation is required.
- Specimen Collection and Transport

See SOP 21120.435 *Specimen Collection and Transport* for procedures for collecting the proper specimens and how to prepare the specimens for transportation to the laboratory.

Specimen Type and Handling

- NP Swab specimens received in saline from clients/sites/central lab are the acceptable specimen type for this assay.
- UTM/VTM specimens are proven stable for

(b) (4)

Extracted RNA should be stored frozen immediately following extraction if not preparing for RT-PCR within (b) (4)

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

REAGENTS AND MATERIALS

Description	Source	Part/Cat No	Storage/Expiration
Extraction			
EasyMAG Lysis Buffer EasyMAG Extraction Buffer 1 EasyMAG Extraction Buffer 2	BioMerieux	280134 280130 280131	Ambient
EasyMAG Extraction Buffer 3	BioMerieux	280132	Unopened: Refrigerated Opened: Ambient
Magnetic Silica	BioMerieux	280133	Refrigerated
NGS Amplification			
Conventional RT-PCR primer mixes	(b) (4)		
(b) (4)			
D5000 ScreenTape	Agilent	5067-5588	Refrigerated
D5000 Sample Buffer	Agilent	5067-5589	Refrigerated
D5000 Ladder	Agilent	5067-5589	Refrigerated
Library Preparation			
Illumina Nextera XT Library Prep Kit - 24 samples (Box 1)	Illumina	FC-131-1024	-20°C
Illumina Nextera XT Library Prep Kit - 24 samples (Box 2)	Illumina	FC-131-1024	Refrigerated
(b) (4)			
Library Sequencing			
(b) (4)			
MiSeq v2 Reagent Kit – (b) (4)	Illumina	MS-(b) (4)	-20ºC
(b) (4)			

QUALITY CONTROL

The quality control program for this test is established in accordance with SOP 21120.517 *Analytical Quality Control: Quality Control Procedures*.

Quality control samples are prepared and control ranges are established and maintained in accordance with SOP 21120.517 *Analytical Quality Control: Quality Control Procedures*.

Extraction Controls

Description	Source	Part/Cat No.	Storage / Expiration*
SARS-CoV-2 Whole Virus Positive Control	(b) (4)		

Description	Source	Part/Cat No.	Storage / Expiration*
(b) (4)			

*: See SOP 21120.380 *Expiration Dating of Laboratory Materials* for proper expiration dating assignment.

Control Procedure

SARS-CoV-2 Positive and Negative controls are included (b) (4)

Whole Virus Positive Control: This control has an approximate concentration of (b) (4)

Negative control:

(b) (4)

Quality Control Acceptance Criteria/Repeat Criteria

Thoroughly document all QC failures or repeat testing on the SARS-CoV-2 NGS Test Record 21120.10121.

Proficiency Testing

Refer to SOP 21120.384 *Proficiency Testing Program* for information on proficiency testing.

EQUIPMENT AND SUPPLIES

Description
Biological Safety Cabinet, Class II
Fixed-angle benchtop centrifuge (with rotor for 2mL tubes)
2mL non-skirted (b) (4) tubes
BioMerieux easyMAG
(b) (4) Thermal Cycler
(b) (4) PCR tubes or (b) (4) Optical 96-well Reaction Plate (standard plate)
(b) (4) caps or Optical Adhesive Covers
(b) (4) swing-bucket centrifuge
Agilent 2200 or 4200 TapeStation and associated components and software
(b) (4) Optical Tube Strips (8x)
(b) (4) Optical Tube Strip Caps (8x)
(b) (4) vortexer
PCR strip tube mini-centrifuge
(b) (4) 96-well MIDI plate magnetic stand
(b) (4) High-speed micro-plate shaker
96-well conical-bottom (b) (4) plate
(b) (4) Sequencer
Refrigerator capable of sustaining 2-8°C
Freezer capable of sustaining -15°C to -35°C
Freezer capable of sustaining -64°C to -90°C

PROCEDURE

Analytical

Nucleic Acid Extractions are performed using the one of the following SOPs:

Confidential Materials. Property of Viracor Eurofins.

A. Refer to 21120.705 *NucliSense easyMAG Total Nucleic Acid Extration* for all instructions for nucleic acid isolations.

1. (b) (4)
2.
3.
4.
5.
6.
7.
8.
9.

Conventional RT-PCR Procedure

Nucleic Acid Amplification is performed according to SOP 21120.461 *Real-Time PCR and RT-PCR Using* (b) (4) (b) (4) *Instruments*.

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

PCR Product Purification: (b) (4) 96-well procedure

(b) (4)

Examples:

(b) (4)

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

DNA Concentration Determination and Quality Check

Note: Purified product DNA should run as a single band or with very minor second band within an agarose gel or other liquid matrix. These matrices will reveal the presence of secondary products and contaminating DNAs and RNAs, but not proteins.

Remove D5000 ScreenTape(s), D5000 Sample Buffer and D5000 Ladder from storage at 2-8°C and allow reagents to equilibrate to room temperature for (b) (4) .

(b) (4)

Refer to *21120.5983 Agilent Tapestation Operation Maintenance and Calibration* for instructions on operation, maintenance, and calibration of the Agilent TapeStation instrument.

(b) (4)

FDA-CBER-2022-1614-1790498

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

(b) (4)

FDA-CBER-2022-1614-1790499

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Nextera XT Library Preparation (Tagmenation and Indexing)

(b) (4)

(b) (4)

Library Purification

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Library Quality Check and Quantification

Remove D5000 ScreenTape(s), D5000 Sample Buffer and D5000 Ladder from storage at 2-8°C and allow reagents to equilibrate to 15-25°C for (b) (4) .

(b) (4)

Library Pooling

(b) (4)

(b) (4)

Library Denaturing and Dilution

(b) (4)

MiSeq Prep and Loading

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

FDA-CBER-2022-1614-1790504

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Run Monitoring

(b) (4)

Post-Run Wash

(b) (4)

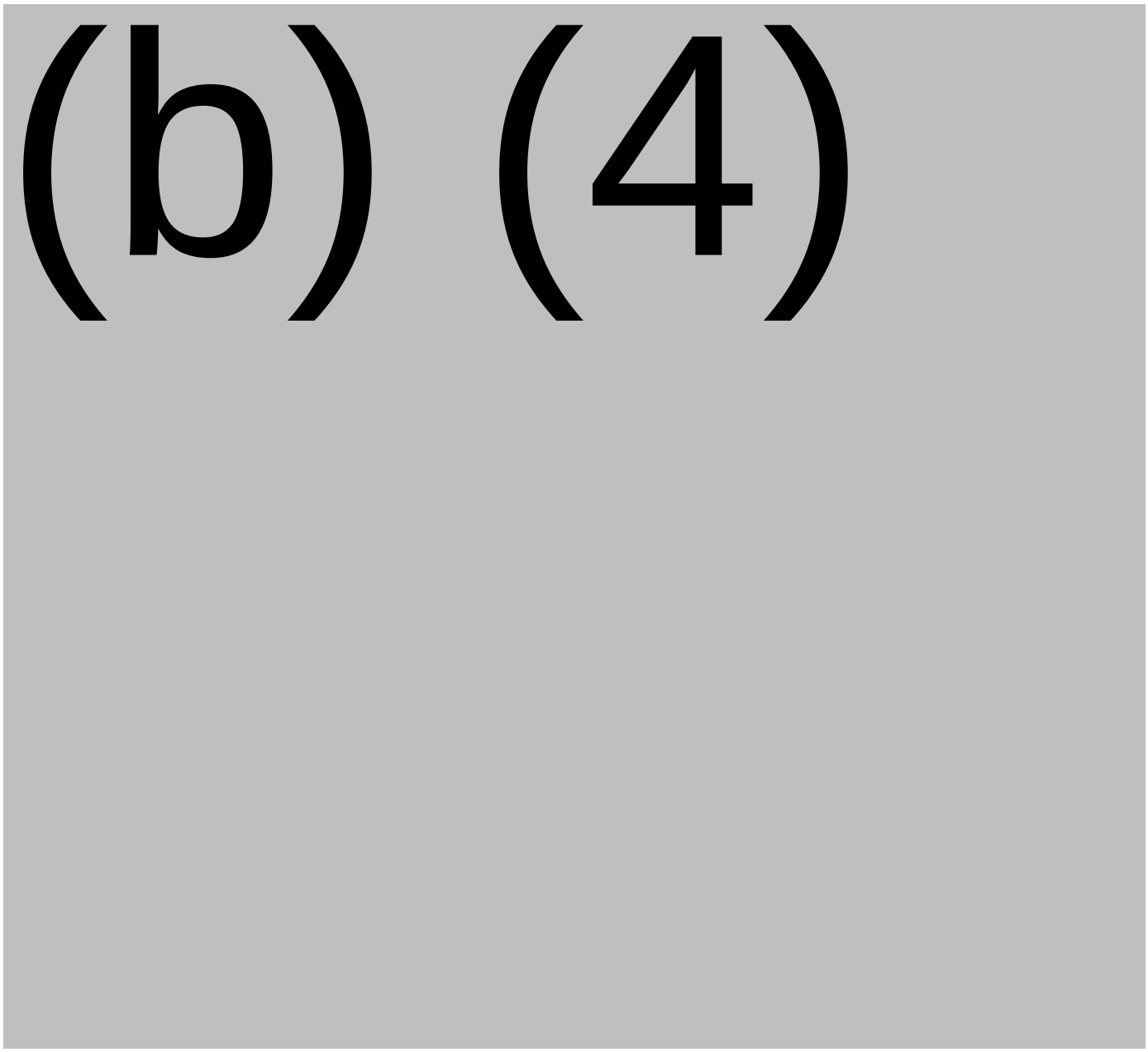
Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Analysis

(b) (4)

Confidential Materials. Property of Viracor Eurofins.



Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Confidential Materials. Property of Viracor Eurofins.

(b) (4)

Notes

(b) (4)

PERFORMANCE SPECIFICATIONS

Not established

SAFETY

The following personal protective equipment will be applied as directed in currently effective Safety SOPS, including but not limited to, gloves, masks, lab coats, face shields and eye protection.

Personnel executing these procedures must be trained on effective Safety SOPs as listed in 21120.265 *Safety Program.*

This procedure requires the use of blood derived from human patients. Follow provisions of SOP 21120.238 *Bloodborne Pathogen Exposure Control Plan* when working with human blood products and disposing of potentially contaminated items.

Avoid contact of reagents with eyes, skin and clothing and follow the procedure in SOP 21120.237 *Chemical Hygiene Plan*.

See Safety Data Sheet (SDS) manual for further details regarding all reagents in the kit(s).

RELATED DOCUMENTS

21120.369 *Analytical Quality Control: Quality Control Procedures*

21120.370 *Analytical Quality Control: Preparation of Control Samples and Establishment of Control Ranges*

21120.384 *Proficiency Testing Program*

21120.705 *NucliSens easyMAG Total Nucleic Acid Extraction*

21120.5983 *Agilent Tapestation Operation Maintenance and Calibration*

21120.443 *Thermal Cycler Preventative Maintenance Operation, Maintenance and Calibration of the* (b) (4) *Thermal Cycler*

21120.7375 *Illumina MiSeq Operation, Maintenance, and Calibration of the Illumina MiSeq /* (b) (4) *Sequencer Analytical Quality Control: Quality Control Procedures*

21120.595 *Specimen Processing Guide*

21120.572 *Verification and Qualification of Critical Laboratory Materials*

21120.764 *Preparation and QC of Oligonucleotides*

21120.10121 SARS-CoV-2 NGS Test Record

21120.10181 SARS-CoV-2 Spike NGS Reagent Log

21120.6292 SARS-CoV-2 Sequencing Assay easyMAG Run Map

REFERENCES

None

Confidential Materials. Property of Viracor Eurofins.