Validation Report for SARS-CoV-2 PriSt MTM

CA-VALRPT-LAB-003
Version number 2.2

CA-VALRPT-LAB-003 Validation Report for SARS-CoV-2 PriSt MTM

Copy of version 2.2 (approved and current)

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Printed By Paven Gahir

Organization PerkinElmer Genomics California

Author
Lynn Deng

Comments for version 2.0 (last major revision)
Added Section 2.3

Comments for version 2.2 (this revision)
Revision history corrected.

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	2/19/2021	2.0	Adam Rosendorff	
Approval	Approval by QA Director	2/19/2021	2.0	Lora J. H. Bean Lora Bean	
Approval	Lab Director	11/23/2020	1.0	Shantelle Lucas Shantelle Lucas	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
2.2	Approved and Current	Minor revision	3/2/2021	3/2/2021	Indefinite
2.2	Retired	Minor revision	3/2/2021	3/2/2021	3/2/2021
2.0	Retired	Major revision	2/19/2021	2/19/2021	3/2/2021
1.0	Retired	Initial version	11/21/2020	11/23/2020	2/19/2021

Approved: 2/19/2021 by Adam Rosendorff

Validation Summary for SARS-CoV-2 Testing using PrimeStoreMTM

CA-VALRPT-CYT-2001

1. PURPOSE

1.1 To provide additional data to validate a molecular diagnostic assay that utilizes real-time polymerase chain reaction (PCR) in order to determine the presences or absences of SARS-CoV-2 RNA in human nasal swabs at CDPH Branch Laboratory, Valencia, CA.

2. SCOPE

2.1 This procedure applies to individuals performing the SARS-CoV-2 Assay at CDPH Branch Laboratory, Valencia, CA.

2.2 This validation report is for SARS-CoV-2 RT-qPCR Reagent kit (Cat # 2019-nCoV-PCR-AUS) and is a supplement to CA-VALRPT-LAB-002.

2.3 This SARS-CoV-2 Assay is approved for each non-waived test for clinical use

3. DEFINITIONS

3.1 IC: An exogenous internal control comprised of TE buffer and bacteriophage MS2, provided in the kit (Cat #: 2019-nCoV-PCR-AUS, tube: nCoV Positive Control)

3.2 Ct or Cq: Cycle number at which PCR response starts to become exponential

3.3 AN: Anterior Nasal

3.4 NSP: Nasopharyngeal

3.5 ORF1ab and N genes: 2 different coding areas in the SARS-CoV2 genome interrogated in this validation using RT-PCR

3.6 FAM, ROX, HEX/VIC: Abbreviations for 3 fluorescent dyes used in reporter oligonucleotides in RT-PCR assay. FAM probe reports on SARS-Cov2 N gene, ROX on ORF1ab gene, and HEX reports on the IC (bacteriophage MS2)

3.7 RT-PCR: real-time PCR

3.8 SARS-CoV-2: Severe acute respiratory syndrome-related coronavirus of the genus Betacoronavirus, strain 2

3.9 PrimerStoreMTM – inactivating molecular transport media used in these studies.

4. ROLES AND RESPONSIBILITIES

4.1 It is the responsibility of the laboratory and delegates to establish any methods required to perform this assay. This must be properly documented in a validation plan, report, standard operating procedures, and any other required documentation.

4.2 All personnel that will perform this assay must understand and comply with established procedures.

4.3 CDPH Branch Laboratory management will provide leadership and support to ensure the development of a properly validated assay in a safe working environment.

4.4 Key Personnel

4.4.1 Clinical laboratory staff at CDPH Branch Laboratory, Valencia, CA

4.4.2 Shantelle Lucas, PhD, HCLD (ABB), CMS, M(ASCP), PHM

4.4.3 Evangeline Voultsis, MS

5. BACKGROUND / CLINICAL SIGNIFICANCE

5.1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) is a betacoronavirus, a novel coronavirus belonging to a family of coronaviruses.

5.2 The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in human specimens collected from individuals suspected of SARS-CoV-2 by their healthcare provider.

5.3 Results are for the identification of SARS-CoV-2 RNA, which is generally detectable in human respiratory specimens during the acute phase of infection Positive results are indicative of 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.

5.4 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.

6. DOCUMENTS AND EQUIPMENT

6.1 Controlled Document Titles and Reference ID that will be used in this verification run:

6.1.1 Accessioning for SARS-CoV-2 Samples (CA-ACC-SOP-001)

6.1.2 Heat Inactivation of Viral Swab Samples (CA-EXT-SOP-001)

6.1.3 Decapping and Batch Preparation for Janus G3 (CA-EXT-SOP-002)

6.1.4 Sample Transfer Using the Janus G3 (CA-EXT-SOP-003)

6.1.5 Viral RNA_DNA Extraction Using the chemagic™ 360-D (CA-EXT-SOP-

004)

6.1.6 SARS-CoV-2 RT-PCR Set-up Using Janus G3 (CA-PCR-SOP-001)

6.1.7 SARS-CoV-2 RT-PCR Using the Analytik Jena (CA-PCR-SOP-002)

6.1.8 SARS-CoV-2 Assay Data Extraction (CA-RPT-SOP-001)

6.1.9 Analysis and Reporting of SARS-CoV-2 Assay (CA-RPT-SOP-002)

6.2 Equipment and Systems with ID

Table 1. Equipment and System IDs

Equipment	Manufacturer	Model	Part/Cat#	Purpose	Environmental Requirements Temperature/ Humidity
chemagic™	PerkinElmer®	360 or 360-D	20240056	DNA/RNA Extraction	Temp: 18 - 35 °C Humidity: < 80 %
Biosafety Hood	Any	Any	Any	Prepare master mix	Dependent on model
JANUS G3	PerkinElmer®	Any	Any	Sample Reformatting and PCR workstation	Temp: 15 – 35 °C Humidity: 60 – 80 %
Microcentrifuge	Any	Any	Any	Collect samples at bottom of well	Dependent on model
Vortex mixer	Any	Any	Any	Mix reagents and/or samples	Dependent on model
Plate Centrifuge	Any	Any	Any	Collect samples at bottom of well	Dependent on model
Pipettes (single and multi-channel) p10, p200, p1000	Any	Any	Any	Pipette reagent and samples	Dependent on model

Validation Summary for SARS-CoV-2 Testing using PrimeStoreMTM

CA-VALRPT-CYT-2001

Table 1. Equipment and System IDs					
Equipment	**Manufacturer**	**Model**	**Part/Cat#**	**Purpose**	**Environmental Requirements Temperature/ Humidity**
Heratherm Oven	Thermo Scientific	Any	Any	Heat samples to kill virus	Temp: 15 - 31 °C Humidity: 10 - 85 %
qPCR Real-Time System	Analytica Jena	AJ384	844-00569-4	RT-PCR	Temp: 15 - 31 °C Humidity: 10 - 85 %

6.3 Reagents, Supplies, and Materials

Table 2. Reagents					
Reagent	**Vendor**	**Manufacturer**	**Part/ Cat#**	**Purpose**	**Storage Conditions**
chemagic™ Viral DNA/RNA 300 Kit H96 component Magnetic Beads B 1 bottle, 150 mL Prep: Ready to use Exp: Unopened, according to the labeled Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Bind RNA/DNA	2 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Lysis Buffer 1 1 bottle, 320 mL Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Lyses cells or other DNA source to get the RNA/DNA into solution	2 - 25 °C

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Table 2. Reagents

Reagent	Vendor	Manufacturer	Part/ Cat#	Purpose	Storage Conditions
chemagic™ Viral DNA/RNA 300 Kit H96 component Binding Buffer 2 1 bottles, 1.3 L Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Create condition to allow the RNA/DNA to bind to the Magnetic beads	2 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Wash Buffer 3 1 bottle, 700 mL Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove non-DNA/RNA contaminants during washing step	2 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Wash Buffer 4 1 bottle, 700 mL Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove non-DNA/RNA contaminants during washing step	2 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Wash Buffer 5 1 bottle, 700 mL Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Remove last traces of non-DNA/RNA contaminates	2 - 25 °C

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Table 2. Reagents

Reagent	Vendor	Manufacturer	Part/ Cat#	Purpose	Storage Conditions
chemagic™ Viral DNA/RNA 300 Kit H96 component Elution Buffer 6 1 bottle, 200 mL TRIS-HCl pH 7.8 - 8.4 Prep: Ready to use Exp: Unopened, according to the label Opened: 30 days	PerkinElmer®	AUS	CMG-1033-S	Elute DNA/RNA	2 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Proteinase K 1 * 11 mL bottle Prep: Reconstituted with 11 mL molecular grade water Exp: Unopened, according to the label Opened: 14 days, 2 - 8 °C	PerkinElmer®	AUS	CMG-1033-S	Added to enhance the efficiency of the lysis step	2 - 8 °C
Molecular Biology Grade Water	Any	Any	Any	Used to reconstitute Proteinase K, NTC control	15 - 25 °C
chemagic™ Viral DNA/RNA 300 Kit H96 component Poly (A) RNA 10 bottles Prep: Reconstituted with 440 µL of Poly (A) RNA Buffer Mix Exp: Unopened, according to label Opened: 4 weeks, 2 - 8 °C	PerkinElmer®	AUS	CMG-1033-S	Added to enhance the efficiency of the lysis step	2 - 8 °C

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Table 2. Reagents

Reagent	Vendor	Manufacturer	Part/ Cat#	Purpose	Storage Conditions
chemagic™ Viral DNA/RNA 300 Kit H96 component Poly (A) RNA Buffer Mix 10 bottles, 2 mL Exp: Unopened, according to label Opened: 30 days.	PerkinElmer®	AUS	CMG-1033-S	Added to Poly (A) RNA to activate.	15 - 25 °C
PrimeStoreMTM – Inactivating molecular transport media	Any	Longhorn vaccines & diagnostics LLC	Variable	Media to create contrived LOD samples	15 - 25 °C
AccuPlexTM SARS-CoV-2 Molecular Controls Kit – Full Genome	SeraCare	SeraCare Life Science, Inc.	0505-0159	Used to create contrived samples	2 - 8 °C
New Coronavirus Nucleic Acid Detection Kit	PerkinElmer®	PerkinElmer®	2019-nCoV-PCR-AUS	RT-PCR kit	-25 to -15°C

Table 3. Materials and Supplies

Material and Supplies	Vendor	Manufacturer	Part/Cat #	Purpose	Storage Conditions
2 mL Deep Well Plate	PerkinElmer®	PerkinElmer®	CMG-555	Load sample/lysis and Elution Buffer B onto the chemagic™ 360	15 - 25 °C
96 Rod Head Disposable Tips	PerkinElmer®	PerkinElmer®	49017-0006	Protect 96 Rod Magnetic Head (on chemagic™ 360) from contamination	15 - 25 °C

Table 3. Materials and Supplies

Material and Supplies	Vendor	Manufacturer	Part/Cat #	Purpose	Storage Conditions
Low-well Plate	PerkinElmer®	PerkinElmer®	CMG-555-1	Load Magnetic Beads B onto the chemagic™ 360	15 - 25 °C
Heat Sealing Foil	Approved vendor	Approved vendor	Approved vendor	Seal plates	N/A
Reagent trough	Any	Any	Any	Preserve reagent stock bottle and allow for multi-channel pipetting	N/A
Tips (10 µL, 200 µL, 1000 µL) compatible with pipettes	Any	Any	Any	Pipette reagent and samples	Dependent on brand
384 Plates for RT-PCR	Approved vendor	Approved vendor	Approved vendor	Hold samples and master mix	N/A
qPCR Seal	Approved vendor	Approved vendor	Approved vendor	Seal 384 well plate	N/A; Expiration Date is written on the box
1.7 mL to 50 mL tube	Any	Any	Any	For master mix preparation	Dependent on brand

6.4 Training Requirements

6.4.1 Personnel that performing this assay will require proper training. This training will include complete review and understanding of all associated documentation and hands-on laboratory training in the performance of this assay. This training will be documented on the Process Training Form.

Validation Summary for SARS-CoV-2 Testing using PrimeStoreMTM **CA-VALRPT-CYT-2001**

7. TESTING PERFORMED

7.1 Limit of Detection (LOD): LOD Range Finding

7.1.1 Contrived samples were created in triplicate. The LODs tested were 10, 20, 40, 60, 120, 180 copies per mL in a total volume of 1 mL per sample. In addition, samples with 0 copy were tested.

7.1.2 Samples were tested both with and without heat inactivation.

7.1.3 A total of 36 samples were run:

7.1.3.1 21 without heat: each LOD in triplicate

7.1.3.2 23 with heat: each LOD in triplicate except 0 copy in 5 replicates.

7.2 Confirmation of LOD

7.2.1 The LOD Confirmation run established the LOD at 120 copies / mL. Samples were created in 20 replicates at 120 copies mL as 1X, and in a level below and above 60 copies / mL and 180 copies /mL.

7.2.2 Due to limited availability of clinical samples, LOD confirmation was performed in PrimeStoreMTM rather than spiked previously tested negative samples.

7.3 Clinical Evaluation

7.3.1 60 previously tested clinical samples (30 samples previously tested as negative and 30 previously tested as positive) were tested. These clinical samples were obtained from a San Mateo County Public Health (CLIA 05D0857622) Laboratory performing SARS-CoV-2 testing.

7.3.2 All previously tested samples were collected in VTM and combined with PrimeStoreMTM in a 1:1 ratio for this study.

7.4 Verification of precision and accuracy

7.4.1 Inter-run Precision: 60 samples were run on different equipments by different technologists.

7.4.2 Intra-run Precision: 12 samples were run in duplicate.

7.4.3 Accuracy: Determined by comparison to previously tested clinical samples.

8. RESULTS

8.1 **LOD Finding (See attached Excel sheet)**

8.1.1 LOD finding studies identified 6/6 expected targets for the *N* and *orf1ab* genes at both 60, 120, and 180 copies / mL.

8.1.2 Based upon LOD finding study 120 copies/mL was chosen as the LOD due to a total of seven N gene dropouts at 60 copies/ml (5/20 copies for non-heated and 2/20 for heated MTM) . In addition, for not heated MTM at 120 copies/mL there was only one drop out of 20 replicates. No drop outs for heated MTM at 120 copies/mL, and at 180 copies/mL all targets were detected.

8.2 **LOD Confirmation (See attached Table 2-Excel sheet)**

8.2.1 Studies as above identified all viral targets (*N* and gene *orf1ab* gene) in all samples at 180 copies / mL.

8.2.2 Note, there was seven dropouts (5 not heated and 2 heated MTM) of the *N* gene target at 60 copies / mL, and one drop out of N gene at 120 copies/mL, confirming the conservative conclusion of 120 copies / mL.

8.3 **Clinical evaluation (See attached Table 3- Excel sheet)**

8.3.1 30 out of 30 previously tested positive samples tested positive.

8.3.2 29 out of 30 previously tested negative samples tested negative.

8.3.3 1 of out 30 previously tested negative samples tested positive; however, this is likely due to the high sensitivity of this.

8.4 **Precision and accuracy**

8.4.1 The 60 previously tested clinical samples (see Clinical Evaluation) were extracted two times (Batch 2.1 and Batch 2.2) on two different equipments and set up by two technologists to show inter-run precision (**Table 4**).

8.4.1.1 Comparing Batch 1 x Batch 2.1 x Batch 2.2 one replicate of COV0203 detected a signal for both *N* and *ORF1ab* genes, in Batch 1 which was discordant with Batches 2.1 and 2.2 (1/180 results)

8.4.1.2 All other results were concordant (179 / 180 results)

8.4.2 Six samples (3 positives and 3 negatives) previously tested clinical samples were tested for intra-run precision (Replicate 3 in Batch 1 and Replicate 4 in Batch 2.1: see **Table 4-Excel sheet**)

8.4.2.1 All but one results within Batches 1 and 2 were concordant

8.4.2.2 Note, sample COV0203, which was discordant in 8.4.1.1 was concordant within this intra-run comparison.

8.4.3 Accuracy was determined by Clinical Evaluation and found to be 59/60 previously tested samples. The one discordant result is likely due to higher sensitivity of this assay (see **Table 3-attached Excel sheet**).

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9. CONCLUSION

9.1 LOD was determined to be 120 copies / mL.

9.2 All samples tested above 1x LOD (120 copies/mL) were detected.

9.3 Clinical evaluation was concordant 59/60 previously tested samples. The one discordant result is likely due to higher sensitivity of this assay.

9.4 Precisions studies across 3 replicates of 60 samples were concordant for 179 / 180 results tested.

9.5 HEX_3 (channel 3) will be used to detect the internal control for this assay.

10. CLINICAL SAMPLE SUMMARY

Total Samples Run	192	
Invalid Samples	0	
Total Analyzable Samples	192	
Total Analyzable Clinical Samples	60	
Number Concordant	59	
Percent Concordant	98.3 %	
Number Discordant	1	
Percent Discordant	1.7 %	
True Positive	100 % (30/30)	
True Negative	96.7 % (29/30)	
False Positive	3.3 % (1/30)	
False Negative	0 % (0/60)	
Sensitivity	100 % (30/30)	TP/(TP+FN)
Specificity	96.7 % (29/30)	TN/(TN+FP)
Clinical Accuracy	99.4% (179/180)	(TP+TN)/[TP+FP+TN+FN]
Clinical Precision	12/12	Inter-run reproducibility

A high percentage of results were concordant with a second laboratory. Of note, all positive results reproduced. Although it is possible for the single discordant result to be false positive, this likely reflects a higher sensitivity of the assay used in this validation or viral load near threshold of detection. Overall, the concordance is within expectations and this validation is accepted by the CDPH Branch Laboratory Directors.

11. APPENDICES

11.1 Appendix A: Evidence of Dr. Lucas's Approval

12. REVISION HISTORY

Version	Summary of Changes	Date
2.2 2.1	Appendix A Added & Revision History updated Inadvertently removed – no changes lost	March 2, 2021
2	Section 2.3 Added	Feb 19, 2021
1	New Document	

APPENDIX A

Evidence of Dr. Lucas's approval on 24Oct2020 t 1:55pm is evidenced by her email to LFS

From: Shantelle Lucas
To: Bean, Lora
Cc: Dowless Kozar, Holli
Subject: [External] FW: CDPH Branch Laboratory Validation
Date: Wednesday, February 24, 2021 11:58:43 AM
Attachments: PrimeStoreMTM_10_24_2020.zip

Use caution when opening links or attachments.

Lora,

See attached.

Shantelle

From: Shantelle Lucas
Sent: Saturday, October 24, 2020 1:55 PM
To: Eleco, Elsa@CDPH <Elsa.Eleco@cdph.ca.gov>; Flores, Elaine@CDPH <Elaine.Flores@cdph.ca.gov>; Thomas, Robert@CDPH <Robert.Thomas@cdph.ca.gov>; Haleh Farzanmehr <hfarzanmehr@genexlpc.com>; Farzanmehr, Haleh@CDPH <Haleh.Farzanmehr@cdph.ca.gov>
Subject: CDPH Branch Laboratory Validation

Hello Elsa, Elaine and Bob,

Attached are the documents for the CDPH Branch Laboratory Validation . Of note, samples only collected in MTM will be used at this time for testing. If you have any questions, please let us know.

Thank you,
Shantelle Lucas