GLOBAL CENTRAL LABS

ANTI-SARS-COV-2 IN SERUM AND PLASMA BY ELECTROCHEMILUMINESCENCE ON ROCHE COBAS 8000 (E602)

PRIMARY VALIDATION SUMMARY

LOCATION

PPD GCL-US
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USA

DEPARTMENT: Chemistry

VALIDATION DOCUMENT NUMBER: VR-GCL-US-2020-06-551

OWNER: (b) (6)

Version number	Issue date	Section affected & Summary of Revision	Name of person Revising
01	04Jun2020	New issue	n/a
02	10Aug2020	Added plasma to Table 10	(b) (6)

Anti-Sars-Cov-2 in Serum and Plasma by Electrochemiluminescence on Roche Cobas 8000 PPD-GCL US	VR-GCL-US-2020-06-551 Issue Date: 10Aug2020

APPROVALS:

Name	Title	Signature/Date
(b) (6)	(b) (6) PPD-GCL	(b) (6) (b) (6) I am the author of this document. Aug 11 2020 8:03 AM -04:00 cosign
(b) (6)	(b) (6) (b) (6) PPD-GCL	(b) (6) (b) (6) I approve this document. Aug 11 2020 10:50 AM -04:00 cosign
(b) (6)	(b) (6) (b) (6) PPD-GCL	(b) (6) (b) (6) I approve this document. Aug 11 2020 1:54 PM -04:00 cosign
Basel Kashlan, MD	Senior Director Laboratory Operations, PPD-GCL	Basel Kashlan Basel Kashlan Medical Director I approve this document. Aug 11 2020 2:09 PM -04:00 cosign
Quality Assurance	QA reviewer	(b) (6) (b) (6) I approve this document. Aug 12 2020 10:50 PM -04:00 cosign

AMENDMENTS TO VALIDATION PLAN PRIOR TO VALIDATION INITIATION

1. Acceptable sample types were updated to include plasma per the manufacturer package insert. The document title was also updated to reflect serum and plasma.
2. Additional modules will be validated at PPD GCL-US and summarized upon completion at a later date.

DEVIATIONS TO VALIDATION PLAN DURING VALIDATION.

1. In order to preserve the volume required for accuracy/ precision samples, correlation was performed with (b) (4)
2. Verification of the assay cut-off index was added in Section 3.

CONCLUSIONS AND RECOMMENDATIONS:

The validation of the Roche electrochemiluminescent kit for the measurement of Anti-Sars-Cov-2 was performed per PPD SOP on validation. The validation parameters showed acceptable assay performance. This validation study has been reviewed, and the performance of the method is considered acceptable for patient testing. For the data that was manually entered, the technologists entered with entry verification performed by a secondary technologist. Signature above signifies acceptance of all data including (b) (4) data and Microsoft Excel calculations. Individual signatures will not be recorded or required on each individual printout.

TABLE OF CONTENTS

1. PURPOSE

The purpose of this document is to demonstrate the performance characteristics of the Roche electrochemiluminescent kit for the measurement of Anti-sars-cov-2 in serum. It includes evaluations of accuracy, precision, cut-off verification, and correlation. Carryover was not assessed as this instrument uses separate pipette tips per sample addition. Analytical sensitivity, maximum dilution, linearity, and reference range were out of the scope of this validation as this assay is qualitative. Analytical specificity and stability were taken from the package insert. This kit is labeled as IVD by the manufacturer.

The validation study was performed by medical technologists at PPD GCL-US facilities. The validation follows the requirements of the Clinical Laboratory Improvement Amendments (CLIA)[1] and College of American Pathologists (CAP),[2] and Food and Drug Administration (FDA)[3].

Data was analyzed by standard statistical procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) using (b) (4) commercial statistical package (version 11.2.0.23) or Microsoft Excel. Acceptability of results throughout the entire validation period were based ≥90% agreement as this assay is qualitative. See Appendix 1 for method performance summary.

2. INTRODUCTION

SARS-CoV-2 is an enveloped, single-stranded RNA virus of the family Coronaviridae, genus Betacoronaviruses. All coronaviruses share similarities in the organization and expression of their genome, which encodes 16 nonstructural proteins and the 4 structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N). Viruses of this family are of zoonotic origin. They cause disease with symptoms ranging from those of a mild common cold to more severe ones such as the Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and Coronavirus Disease 2019 (COVID-19). Other coronaviruses known to infect humans include 229E, NL63, OC43 and HKU1. The latter are ubiquitous, and infection typically causes common cold or flu-like symptoms. Additional information can be found in the manufacturer's package insert as referenced in Section 15.

[1] Federal Register 2003 (April 24): 7164 [42CFR493.1253]
[2] College of American Pathologists (CAP) All Common Checklist, COM.40000 - 50100
[3] FDA Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human services, May 2018.

2.1 TEST PRINCIPLE

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, biotinylated SARS-CoV-2-specific recombinant antigen and SARS-CoV-2-specific recombinant antigen labeled with a ruthenium complex form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

3. REAGENTS AND EQUIPMENT

Table 1: Reagents

Name	Manufacturer	Catalog #	Lot #/ Expiration Date
Elecsys Anti-SARS-Cov-2	Roche Diagnostics	(b) (4) (b) (4)	(b) (4) (b) (4)
Positive Donor Samples	BioIVT	N/A	N/A
Asymptomatic Donor Samples (assumed negative)	Laboratory Donors	N/A	N/A

Table 2: Instruments and Equipment

Location	Name	Manufacturer	Serial No.
PPD GCL-US	Roche Cobas 8000 e602	Roche Diagnostics	(b) (4)

Table 3: Result Interpretation/ Cut-Off Verification

COI	Result	Interpretation
<1.0	Non-Reactive	Negative
≥1.0	Reactive	Positive

(b) (4)

Test Date	(b) (4)	(b) (4)	Pass/Fail?
18May2020	(b) (4)	(b) (4)	Pass

Decimal places are dependent on the sample value on the Roche Cobas Immunoassay platform, as such different decimals are reflected at different sample values.

4. ACCURACY

(b) (4)

Inaccuracy was verified by replicate analysis of known negative and positive samples obtained from laboratory donors and BioIVT. Intra-assay and inter-assay inaccuracy were calculated at each level using data from each of the (b) (4) assay runs at the concentrations listed below.

Table 4: Inaccuracy and Imprecision Sample Preparation

Experiment Samples	COI	Target Interpretation	Total Volume (uL)	Volume per sample (uL)	# Replicates per sample
(b) (4)					

From the observations, the conclusions will be compared to the expected conclusions, the % agreement will be calculated across all samples and replicates using Microsoft Excel.

$$\%agreement = \frac{Correct\ interpretations}{Total\ interpretations} * 100\%$$

ACCEPTABILITY: % agreement is ≥90%, cutoff value samples may be showing values around (b) (4) agreement due to variability on the method, and this is acceptable.

CONCLUSION: Accuracy PASSED. Inter and Intra-assay accuracy were verified across the tested range. See data in Tables 5-6.

Table 5. Accuracy/Precision PPD GCL-US (b) (4)

Method Verification:	Accuracy/Precision (b) (4)
Sample Matrix:	Serum
Analyst:	(b) (6)
Dates:	28May2020-02Jun2020
Units:	Qualitative (Negative, Positive)
Acceptance Criteria:	≥90% Agreement

Test Date	(b) (4)				
	(b) (4)				
	Pass/Fail	PASS	PASS	PASS	PASS	PASS

A new reagent lot was implemented 01Jun2020. Although COI values vary between (b) (4) all samples returned the same qualitative interpretation. A reagent lot crossover was performed per PPD SOP and was acceptable per SOP defined criteria as all results met 100% agreement.

Table 6. Accuracy/Precision PPD GCL-US (b) (4)

Method Verification:	Accuracy/Precision (b) (4)
Sample Matrix:	Serum
Analyst:	(b) (6)
Dates:	28May2020-02Jun2020
Units:	Qualitative (Negative, Positive)
Acceptance Criteria:	≥90% Agreement

Test Date	(b) (4)				
	(b) (4)				
	Pass/Fail	PASS	PASS	PASS	PASS	PASS

A new reagent lot was implemented 01Jun2020. Although COI values vary between (b) (4) all samples returned the same qualitative interpretation. A reagent lot crossover was performed per PPD SOP and was acceptable per SOP defined criteria as all results met 100% agreement.

5. CORRELATION

(b) (4)

(b) (4)

The % agreement will be determined using (b) (4) or excel. The data will be shown in concordance tables. The % agreement is calculated as:

$$\%agreement = \frac{Correct\ interpretations}{Total\ interpretations} * 100\%$$

ACCEPTABILITY: % agreement between methods should be ≥90%.

CONCLUSION: Correlation PASSED. See Table 7 for data and Appendix 2 for EPE reports.

Table 7. Correlation PPD GCL-US (b) (4)

Method Verification:	(b) (4)						
Sample Matrix:	Serum						
Analyst:	(b) (6						
Dates:	(b) (4)						
Units:	Qualitative (Negative, Positive)						
Acceptance Criteria:	≥ 90% Agreement						
Sample	**Material**	**X (PPD-US (b) (4)**		**X (PPD-US (b) (4)**		**% Agreement**	**Pass/ Fail**
		COI	**Interpretation**	**COI**	**Interpretation**		

(b) (4)

6. MATRIX EFFECT

Permitted clinical samples are restricted to the manufacturer approved specimen collection types shown in the package insert and unadulterated human samples otherwise not pre-treated with chemical modifiers such as acids, bases, detergents, or ionic agents prior to analysis. Evaluation of matrix effect was out of scope of this validation.

Serum, lithium heparin, K2EDTA, and K3EDTA are acceptable matrices per manufacturer.

7. PRECISION

(b) (4)

Imprecision was determined by observation of the scatter of individual measures when the analytical method is performed repeatedly to multiple aliquots of the sample.

Intra-assay and inter-assay imprecision were determined using the accuracy samples and experimental design described in section 4.

ACCEPTABILITY: As a qualitative assay, assay's precision is acceptable if multiple replicates of each sample demonstrate ≥90% consistency in terms of qualitative interpretation.

CONCLUSION: Imprecision PASSED. Intra-assay and inter-assay imprecision were verified across the tested range. See Tables 5-6 in Section 4 for data and Appendix 3 for (b) (4) reports. As this assay is qualitative, precision is for information purposes only.

8. ANALYTICAL SE SITIVITY (LLOQ)

Given the qualitative nature of the method, LLoQ was out of the scope of this validation.

9. ANALYTICAL SPECIFICITY

Analytical specificity was taken from manufacturer's package insert. The following interferents do not have a significant effect on assay results up to the concentrations listed in Table 8.

Table 8: Interferences as tested by the manufacturer

Analyte	Biotin Interference
Anti-sars-cov-2	≤4912 nmol/L or ≤1200 ng/mL

Interferences for hemolysis, bilirubin, and rheumatoid factor and pharmaceutical compounds other than biotin have not been tested by the manufacturer as such, samples that are grossly hemolyzed, icteric, or lipemic are not permitted.

10. ANALYTICAL MEASUREMENT RANGE (LINEARITY & ULOQ)

Given the qualitative nature of the method, AMR/ Linearity was out of the scope of this validation.

11. MAXIMUM DILUTION

Given the qualitative nature of the method, maximum dilution was out of the scope of this validation.

12. CARRYOVER

The assay requires separate pipette tips per sample addition, as such carryover does not exist.

13. REFERENCE RANGE

Reference range is by default negative and was out of the scope of this validation.

14. STABILITY

Stability of samples has been established by the manufacturer and is cited below.

Table 9: Stability Conditions

Stability Sample Type	Storage Condition	Time
Patient Samples, serum/plasma	Ambient (20±5°C)	3 Days[4]
Patient Samples, serum/plasma	Refrigerated (5 ± 3°C)	7 Days[4]
Patient Samples, serum/plasma	Frozen (-20 ± 10°C)	28 Days[4]
Patient Samples, serum/plasma	Frozen (-70 ± 10°C)	28 Days (considered same as -20 ± 10°C)[3]
Patient Samples, serum/plasma	Freeze-Thaw cycles	2[4]

[4] Elecsys Anti-Sars-Cov-2. 09203095190. 2020-04. V1.0.

15. REFERENCES

1. Federal Register 2003 (April 24): 7164 [42CFR493.1253]
2. College of American Pathologists (CAP) All Common Checklist, COM.40000 – 50100
3. FDA Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human services, May 2018.
4. Elecsys Anti-Sars-Cov-2. 09203095190. 2020-04. V1.0.
5. GCL-LAB-1044r00 Primary Reagent and Calibrator Lot to Lot Comparison and Acceptability

APPENDIX 1: METHOD PERFORMANCE SUMMARY

ACCEPTABLE SAMPLE TYPES

MATRIX	COLLECTION TUBE TYPE
Serum	Standard separating tube with or without gel
Plasma	Lithium heparin, K2EDTA, K3EDTA

Total Allowable Error (TaE): ≥90% Agreement Source Reference: CLSI
CAP Method Classification (IVD, LDT, RUO): IVD
Conventional Units of measure: Qualitative (Negative, Positive)

PERFORMANCE SUMMARY

PERFORMANCE MEASURE		PERFORMANCE TO BE USED IN SOP/MTM	SOURCE (PI/VR)	DOCUMENT SECTION
Accuracy		(b) (4)	VR	4
Correlation (n = (b) (4) samples)		Comparator: PPD GCL US (b) (4) Alternative: PPD GCL US (b) (4) % Agreement: 100%	VR	5
Matrix Effect		N/A	PI	6
Precision		% Agreement: 100%	VR	4/7
Analytical Sensitivity (LLOQ)		N/A	VR	8
Analytical Specificity (Interferences)		Biotin: ≤4912 nmol/L or ≤1200 ng/mL Grossly hemolyzed, lipemic, or icteric samples are not permitted	VR	9
Analytical Measuring Range		N/A- Assay reported as Negative or Positive	VR	10
Reportable Range		N/A- Assay reported as Negative or Positive	VR	11
Maximum Dilution		N/A	VR	11
Reference Range (All)		Negative	VR	13
Stability	Ambient (20±5°C)	3 Days	VR	14
	Refrigerated (5±3°C)	7 Days	VR	
	Frozen (-20±10°C)	28 Days	VR	
	Frozen (-70±10°C)	28 Days	VR	
Freeze-Thaw		2	VR	14

APPENDIX 2. CORRELATION

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APPENDIX 3. IMPRECISION

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