Elecsys® Anti-SARS-CoV-2 Immunoassay

for use with **cobas e** 411, **cobas e** 601, **cobas e** 602 and **cobas e** 801 systems

For use under the Emergency Use Authorization (EUA) only

Assay Verification Proposal

The **Elecsys®** Anti-SARS-CoV-2 is an immunoassay for the *in vitro* qualitative detection of antibodies (including IgG) to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in human serum and plasma. The test is intended as an aid in the determination of the immune reaction to SARS-CoV-2.

The World Health Organization (WHO) has declared a Public Health Emergency of International Concern (PHEIC) in response to the COVID-19 (novel coronavirus) outbreak. This is a serious health situation and we recognize that the public and private sectors across the globe need to work together to help effectively manage this developing situation. Roche is working with health authorities and the governments in affected countries to ensure people can get access to screening and healthcare.

As a leader in diagnostics, Roche is committed to providing testing solutions for the world's most challenging healthcare emergencies. We are committed to delivering as many tests as possible within the limits of supply. The following verification proposal was developed by Roche Medical and Scientific Affairs to simplify the verification process of the **Elecsys®** Anti-SARS-CoV-2 test, providing recommendations for implementation in a manner that conserves resources and allows as many laboratories as possible to get up and running in the shortest period of time without compromising assay quality.

Given the limited supply of reagents and the prioritization of patient testing, this proposal provides assay verification guidance to laboratories that allows for adherence to quality assurance procedures while conserving test material. Our goal is to support as many laboratories as possible and to provide the greatest access to testing for patients in need while meeting the CAP/CLIA/Joint Commission regulatory requirements for assay verification (see justification below).

Verification of performance specifications

> *"The laboratory is required to check (verify) the manufacturer's performance specification provided in the package insert for accuracy, precision, reportable range and reference ranges for each new unmodified, moderate to high complexity test that the laboratory performs before reporting test results. The verification process helps ensure the test, when used in your laboratory by your testing personnel for your patient population, is performing as the manufacturer intended. This requirement applies when the laboratory replaces a test system or instrument with the same model or a different model, adds new tests, or changes the manufacturer of a test kit."*[1,2]

> *"Verifying a test system's accuracy, precision and reportable range may be performed using the same samples. For example, you may test samples with known values at the upper and lower end of the manufacturer's reportable range, along with samples that are in the normal range for your patient population, in different runs and on different days, using several of the personnel who will normally perform the testing. The activities of the personnel verifying the test system will also facilitate meeting CLIA's personnel training/competency requirements for these employees. In addition, the Laboratory Director may use the verification process to meet the CLIA requirements for establishing the test system's quality control protocol, an essential component of the laboratory's overall quality system."*[1,2]

Per CLIA regulation and CAP guidelines[1-4] for Qualitative testing (moderate and high complexity tests)

- the laboratory must verify accuracy of a method by being able to identify the presence/absence of the analyte. The laboratory needs to compare the accuracy of the test results it obtains when using a test system with the manufacturer's accuracy claims. This can be done by testing commercially available calibrators/calibration or quality control (QC) materials with known values, proficiency testing materials that have established values, or previously tested patient specimens with established values

- the laboratory must verify precision by repeatedly testing the same samples under different conditions and get the same or comparable results (reproducible), regardless of which member of the laboratory's testing personnel performs the test (operator variance). For example, consider testing samples with known values (e.g. previously reported patient samples, QC materials, or calibration materials) in different runs on different days, as well as multiple times within the same run. Several of the laboratory's testing personnel should participate in this evaluation to help determine overall laboratory variance. ***Exception: For fully automated test systems that are not operator dependent, operator variance should not affect the test's precision and may not need to be evaluated by more than one person***

- the laboratory must verify the manufacturer's established reportable range for the test by choosing samples (e.g. previously reported patient or proficiency testing samples with abnormal high and abnormal low values, QC materials, or calibration materials) with known values at the different levels the manufacturer claims accurate results can be produced by the test system

Laboratories using Emergency Use Authorization (EUA) Assays[7]

CAP-accredited laboratories can obtain EUA kits from authorized manufacturers and verify test performance to perform testing in their own laboratories. To perform testing and report patient test results, a laboratory must:

1. Ensure that personnel are trained and qualified to perform testing based on the specific level FDA authorization received for the test in the EUA Letter of Authorization.

2. Follow the assay or test system's protocol as authorized by the US Food and Drug Administration (FDA) without modification.

3. Verify test method performance specifications on site following the CAP's All Common Checklist requirements COM.40300, COM.40475, and COM.40500.

4. Update the laboratory's CAP activity menu in Organization Profile by logging into e-Lab Solutions Suite on cap.org.

The Verification Proposal contained in this document describes experiments to verify accuracy and precision.

Assay verification steps

Laboratory objective: *Verify Roche EUA package insert specifications meet CLIA minimum requirements.*

- Positive and Negative leftover plasma/serum samples from patients with or without SARS-CoV-2 infection can be used to establish performance characteristics; It is suggested that positive patient samples are at least 14 days post diagnosis of infection
- Data is generated over 2 runs: one run of 10-20 positive and 10-20 negative patient samples run by the designated laboratory operator; one run of pooled positive and pooled negative patient samples (see description below)
- Data collected by the laboratory operator can be placed into provided spreadsheet for analysis
- The test process outlined below is based on the minimum guidelines specified by CAP and CLIA

NOTE: The final verification plan and documentation of verification data is the responsibility of the laboratory

Verification Run

Verification Runs should be completed after properly calibrating the assay as well as testing positive and negative controls NOTE: Calibrators are required and provided (ACOV2Cal 1 (negative) and ACOV2Cal 2 (positive). Controls are required but not provided with the test kit and must be prepared by the laboratory (see information in control development section).

The verification run will consist of two runs:
Run 1: 10-20 positive and 10-20 negative samples.
Run 2: Precision run of pooled positive and pooled negative samples run in 10 replicates for a total of 20 samples

Precision Run Samples
Using at least 10 samples of positive samples or negative samples from Run 1, pool together and then dilute to the desired COI value (COI=Cut off index)

See layout below.

Verification Run 1

Samples	Sample Type	Sample ID	Results	
			Expected	Observed
1	Anti-SARS-CoV-2 Pos Patient 1	SARS-CoV-2 1	Pos	
2	Anti-SARS-CoV-2 Pos Patient 2	SARS-CoV-2 2	Pos	
3	Anti-SARS-CoV-2 Pos Patient 3	SARS-CoV-2 3	Pos	
4	Anti-SARS-CoV-2 Pos Patient 4	SARS-CoV-2 4	Pos	
5	Anti-SARS-CoV-2 Pos Patient 5	SARS-CoV-2 5	Pos	
6	Anti-SARS-CoV-2 Pos Patient 6	SARS-CoV-2 6	Pos	
7	Anti-SARS-CoV-2 Pos Patient 7	SARS-CoV-2 7	Pos	
8	Anti-SARS-CoV-2 Pos Patient 8	SARS-CoV-2 8	Pos	
9	Anti-SARS-CoV-2 Pos Patient 9	SARS-CoV-2 9	Pos	
10	Anti-SARS-CoV-2 Pos Patient 10	SARS-CoV-2 10	Pos	
11	Anti-SARS-CoV-2 Pos Patient 11	SARS-CoV-2 11	Pos	
12	Anti-SARS-CoV-2 Pos Patient 12	SARS-CoV-2 12	Pos	
13	Anti-SARS-CoV-2 Pos Patient 13	SARS-CoV-2 13	Pos	
14	Anti-SARS-CoV-2 Pos Patient 14	SARS-CoV-2 14	Pos	
15	Anti-SARS-CoV-2 Pos Patient 15	SARS-CoV-2 15	Pos	
16	Anti-SARS-CoV-2 Pos Patient 16	SARS-CoV-2 16	Pos	
17	Anti-SARS-CoV-2 Pos Patient 17	SARS-CoV-2 17	Pos	
18	Anti-SARS-CoV-2 Pos Patient 18	SARS-CoV-2 18	Pos	
19	Anti-SARS-CoV-2 Pos Patient 19	SARS-CoV-2 19	Pos	
20	Anti-SARS-CoV-2 Pos Patient 20	SARS-CoV-2 20	Pos	
21	Anti-SARS-CoV-2 Neg Patient 1	SARS-CoV-2 21	Neg	
22	Anti-SARS-CoV-2 Neg Patient 2	SARS-CoV-2 22	Neg	
23	Anti-SARS-CoV-2 Neg Patient 3	SARS-CoV-2 23	Neg	
24	Anti-SARS-CoV-2 Neg Patient 4	SARS-CoV-2 24	Neg	
25	Anti-SARS-CoV-2 Neg Patient 5	SARS-CoV-2 25	Neg	
26	Anti-SARS-CoV-2 Neg Patient 6	SARS-CoV-2 26	Neg	
27	Anti-SARS-CoV-2 Neg Patient 7	SARS-CoV-2 27	Neg	
28	Anti-SARS-CoV-2 Neg Patient 8	SARS-CoV-2 28	Neg	
29	Anti-SARS-CoV-2 Neg Patient 9	SARS-CoV-2 29	Neg	
30	Anti-SARS-CoV-2 Neg Patient 10	SARS-CoV-2 30	Neg	
31	Anti-SARS-CoV-2 Neg Patient 11	SARS-CoV-2 31	Neg	
32	Anti-SARS-CoV-2 Neg Patient 12	SARS-CoV-2 32	Neg	
33	Anti-SARS-CoV-2 Neg Patient 13	SARS-CoV-2 33	Neg	
34	Anti-SARS-CoV-2 Neg Patient 14	SARS-CoV-2 34	Neg	
35	Anti-SARS-CoV-2 Neg Patient 15	SARS-CoV-2 35	Neg	
36	Anti-SARS-CoV-2 Neg Patient 16	SARS-CoV-2 36	Neg	
37	Anti-SARS-CoV-2 Neg Patient 17	SARS-CoV-2 37	Neg	
38	Anti-SARS-CoV-2 Neg Patient 18	SARS-CoV-2 38	Neg	
39	Anti-SARS-CoV-2 Neg Patient 19	SARS-CoV-2 39	Neg	
40	Anti-SARS-CoV-2 Neg Patient 20	SARS-CoV-2 40	Neg	

Verification Run 2

Samples	Sample Type	Sample ID	Results	
			Expected	Observed
1	Anti-SARS-CoV-2 Pooled Positive 1	SARS-CoV-2 1	Pos	
2	Anti-SARS-CoV-2 Pooled Positive 2	SARS-CoV-2 2	Pos	
3	Anti-SARS-CoV-2 Pooled Positive 3	SARS-CoV-2 3	Pos	
4	Anti-SARS-CoV-2 Pooled Positive 4	SARS-CoV-2 4	Pos	
5	Anti-SARS-CoV-2 Pooled Positive 5	SARS-CoV-2 5	Pos	
6	Anti-SARS-CoV-2 Pooled Positive 6	SARS-CoV-2 6	Pos	
7	Anti-SARS-CoV-2 Pooled Positive 7	SARS-CoV-2 7	Pos	
8	Anti-SARS-CoV-2 Pooled Positive 8	SARS-CoV-2 8	Pos	
9	Anti-SARS-CoV-2 Pooled Positive 9	SARS-CoV-2 9	Pos	
10	Anti-SARS-CoV-2 Pooled Positive 10	SARS-CoV-2 10	Pos	
11	Anti-SARS-CoV-2 Pooled Negative 1	SARS-CoV-2 11	Neg	
12	Anti-SARS-CoV-2 Pooled Negative 2	SARS-CoV-2 12	Neg	
13	Anti-SARS-CoV-2 Pooled Negative 3	SARS-CoV-2 13	Neg	
14	Anti-SARS-CoV-2 Pooled Negative 4	SARS-CoV-2 14	Neg	
15	Anti-SARS-CoV-2 Pooled Negative 5	SARS-CoV-2 15	Neg	
16	Anti-SARS-CoV-2 Pooled Negative 6	SARS-CoV-2 16	Neg	
17	Anti-SARS-CoV-2 Pooled Negative 7	SARS-CoV-2 17	Neg	
18	Anti-SARS-CoV-2 Pooled Negative 8	SARS-CoV-2 18	Neg	
19	Anti-SARS-CoV-2 Pooled Negative 9	SARS-CoV-2 19	Neg	
20	Anti-SARS-CoV-2 Pooled Negative 10	SARS-CoV-2 20	Neg	

It is recommended:

For each instrument being used for testing, the verification run should be performed.

These configurations are set up to meet the performance specifications required (accuracy and precision) including within-run variability. For between-run variability assessment, it is recommended to track the kit controls for validity over time per CAP guidance. [4,6]

Data Analysis

Data will be analyzed and performance assessed by utilizing a 2x2 table identifying TP (true positive), FP (false positive), TN (true negative) and FN (false negative) for the method compared to the expected results of the panel member for calculation of overall percent agreement, positive percent agreement, negative percent agreement, negative predicative value and positive predicative value. See attached spreadsheet.

Control Development

For quality control, use controls prepared as follows:

Negative control:

Determine the COI of ACOV2 Cal1 by measuring it as a routine sample. Pool serum samples with a COI result of ≤ 150% compared to the COI result of ACOV2 Cal1 (pooling of ≥ 5 non-reactive samples in this range is recommended). Mix carefully, avoiding foam formation. Prepare aliquots of at least 250 µl from this sample pool and store frozen at -20 ± 5°C or colder. Use these aliquots to perform regular quality control. This negative control has a target value range of COI < 0.8 (qualitative assay result "non-reac.").

Positive control:

Determine the COI of ACOV2 Cal2 by measuring it as a routine sample. Pool serum samples with a COI result that is equal or higher than the COI result of ACOV2 Cal2 (pooling of ≥ 3 reactive samples in this range is recommended). Dilute the sample pool by adding pooled negative serum (pooling criterion see negative control) or Diluent MultiAssay to obtain a COI between 3 and 15. Mix carefully, avoiding foam formation. It is recommended to confirm calculated reactivity after dilution by a measurement. Prepare aliquots of at least 250 µl from this sample pool and store frozen at -20 ± 5°C or colder. Use these aliquots to perform regular quality control.

NOTE: Upon first use of this control, determine the COI of the control by measurement of the control in triplicate and using a freshly opened and calibrated reagent rack pack. The obtained median of these measurements serves as the target value for this positive control.

SEE Package Insert for more details.[8]

Sample Handling

Per CDC guidance[5] for laboratories working with potentially infectious materials,

- Laboratory workers should wear appropriate personal protective equipment (PPE) which includes disposable gloves, laboratory coat/gown and eye protection when handling potentially infectious specimens.
- Any procedure with the potential to generate aerosols or droplets (e.g., vortexing) should be performed in a certified Class II Biological Safety Cabinet (BSC). Appropriate physical containment devices (e.g., centrifuge safety buckets; sealed rotors) should be used for centrifugation. Ideally, rotors and buckets should be loaded and unloaded in a BSC. For any procedures outside of a BSC, eye and face protection (e.g., goggles, mask, face shield) or other physical barriers (e.g., splash shield) should be used to minimize the risk of exposure to laboratory staff.
- After specimens are processed, decontaminate work surfaces and equipment with appropriate disinfectants. Use EPA-registered hospital disinfectants with label claims to be effective against other respiratory pathogens, such as seasonal influenza and other human coronaviruses. Follow manufacturer's recommendations for use – dilution (i.e., concentration), contact time, and care in handling.
- For SARS-CoV-2 laboratory waste, follow standard procedures associated with other respiratory pathogens, such as seasonal influenza and other human coronaviruses.
- **For diluted or aliquoted specimens,** take precautions as stated above and in a certified Class II BSC in a BSL-2 facility. Site- and activity-specific risk assessments should be performed to determine if enhanced biosafety precautions are warranted based on situational needs (e.g., high testing volumes)

References

1. CLIA Verification of Performance Specifications, Brochure #2, February 2004.
2. State Operations Manual Appendix C- Interpretive Guidelines CFR493.1253: www.cms.hhs.gov/clia.
3. CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition*. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
4. College of American Pathologists. All Commons Checklist 2019. Test Method Validation and Verification-Nonwaived tests pgs 43-46.
5. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html
6. CAP Guidance letter for Laboratories using EUA Assays. March 9, 2020. Letter on file.
7. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations
8. Elecsys Anti-SARS-CoV-2 Package Insert, Roche Diagnostics 2020.