TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. HISTORICAL REVISION SUMMARY

Table 1. Chronological Summary of Previous Versions

Version #	Effective Date	Originator	Summary
3.0	Current	(b) (6)	Updated Repeat process, added guidance for QCs expiry dates, updated data export process to include use of a USB Sharing Device.
2.0	25-Sep-2020	(b) (6)	Updated matierals and equipment, created sections in procedure, added figures, added primary method of running clinical samples, added procedure for use of VRD LabWare LIMS, Removed startup and shutdown procedure, added repeat process procedure, added steps to the data export, added steps to the skills assessment.
1.0	29-Jul-2020	(b) (4)	New SOP

2. PURPOSE

This document describes the procedures used to perform the Elecsys anti SARS-CoV-2 assay using the Roche Diagnostic cobas(b) (4) Immunoassay System. The Roche Diagnostic cobas(b) (4) Immunoassay System is a fully automated, random access, software-controlled system for immunoassay analysis.

3. SCOPE

Personnel in Vaccines Research and Development that perform regulated testing are required to follow this SOP.

Table 2. Vaccine Research Functional Units

Functional Units	Location
VRD (Vaccine Research and Development)	Pearl River

4. GLOSSARY

Table 3. Terms and Definitions

Term	Definition
ACOV2 Cal1	Negative calibrator 1
ACOV2 Cal2	Positive calibrator 2
Coated Microspheres	Streptavidin coated beads at 0.72mg/mL
COVID-19	Coronavirus Disease 2019
Elecsys Anti-SARS-CoV-2	An immunoassay intended for qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma
LIMS	Laboratory Information Management System

Table 3. Terms and Definitions

Term	Definition
mAb	Monoclonal antibody (also known as capture antibodies)
QCS	Quality Control Samples
Room Temperature (RT)	Room temperature in this SOP is defined as 20°C with a range of 18-25°C.
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SM	Sample Management

5. GENERAL

SARS-CoV-2 is an enveloped, single-stranded RNA virus of the family Coronaviridae, genus Betacoronavirus. 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). 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).

Elecsys Anti-SARS-CoV-2 is an immunoassay intended for qualitative detection of antibodies to SARS-CoV-2 in human serum and plasma (K2-EDTA, K3-EDTA, Li-heparin). The assay uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV-2. The test is intended for use as an aid in identifying individuals with an immune response to SARS-CoV-2 which indicates a recent or prior infection. Antibodies to SARS-CoV-2 are generally detectable in blood for several days after the initial infection.

6. RESPONSIBILITIES

- All personnel and positions referred to in this procedure are considered to have an alternate.
- An alternate must ensure that they are trained and knowledgeable with the process.

Table 4. Roles and Responsibilities

Role	Responsibilities
Laboratory Analyst	• Performs the test method following the procedures described in this SOP. • Reads, understands, and completes training on VR-SOP-FE-11297 (SA01). • Completes documentation of the work performed and any deviations that occurred. • Follows applicable departmental SOPs regarding safety, equipment use and documentation practices. • Initiates LDRs, if needed. • Notifies supervisor of any atypical events. • This person cannot be the Data Reviewer for the same batch.

Table 4. Roles and Responsibilities

Role	Responsibilities
Data Reviewer	• Reads, understands, and completes training on VR-SOP-LC-11296 (SA01). • Reviews documentation to ensure completeness of data entry and compliance with Pfizer policies. Directs the Laboratory Analyst to correct documentation errors. • This person cannot be the Laboratory Analyst for the same batch. • Initiates LDRs, if needed.
Supervisor	• Reviews and signs documentation. • Ensures that analysts have been trained on appropriate SOPs. • Ensures LDRs are initiated and properly investigated. • Ensures that equipment and instruments have been properly calibrated and maintained.

7. SAFETY

- Biosafety level 2 precautions should be taken when handling human and non-human biological fluids or materials as recommended in the CDC/NIH manual, "Biosafety in Microbiological and Biomedical Laboratories", 5th ed., 2009: http://www.cdc.gov/biosafety/publications/bmbl5/.

- Personnel must be trained on current laboratory safety procedures and made aware of the Material Safety Data Sheets for chemicals being used, before performing this procedure.

- All laboratory work is to be carried out in compliance with site safety policies. Refer to the Global Laboratory Safety Reference Manual: http:// (b) (4) or the Pearl River Environmental Health and Safety Laboratory Manual: http:// (b) (4) for safety precautions.

- Lab coats, safety glasses and disposable gloves must be worn at all times when handling human specimens.

7.1. Equipment

- Roche cobas (b) (4) analyzer, (b) (4), or equivalent.
- Scientific freezers, -80°C ±15°C.
- Scientific refrigerator, 5°C (2°C to 8°C).
- (b) (4) Centrifuge, (b) (4) or equivalent.
- (b) (4) Capper, (b) (4).
- (b) (4) Thawing Station, (b) (4).

- (b) (4), or equivalent.
- Computer.
- Printer.
- (b) (4) Peripheral Sharing Switch, (b) (4), or equivalent.

7.2. Materials

- 11706802001, AssayCup, 60 x 60 reaction cups.
- 11706799001, AssayTip, 30 x 120 pipette tips.
- 11800507001, Clean-Liner.
- Polypropylene pipette tips, various sizes.
- Sterile disposable serological pipettes, various sizes.
- Biohazardous waste receptacle, biohazardous sharps waste container.

7.3. Reagent Stocks

- Elecsys Anti-SARS-CoV-2 assay kit, Roche, Cat # 09203095190.
- Molecular Biology Grade Water, (b) (4), or equivalent.

7.4. Negative and Positive Calibrators

- ACOV2 Cal1 Negative calibrator 1 (white cap), 1 bottle of 0.67 mL: Human serum, non-reactive for anti-SARS-CoV-2 antibodies; buffer; preservative.
- ACOV2 Cal2 Positive calibrator 2 (black cap), 1 bottle of 0.67 mL: Human serum, reactive for anti-SARS-CoV-2 antibodies; buffer; preservative.

7.5. Negative and Positive Quality Controls

- COV2-Negative Serum.
- COV2-Positive Serum.
- PreciControl Anti-SARS-COV-2 (PC ACOV2 1), Negative Control, 1 yellow bottle of 1.0 mL, Cat# 09216928190.
- PreciControl Anti-SARS-COV-2 (PC ACOV2 2), Positive Control, 1 brown bottle of 1.0 mL, Cat# 09216928190.

7.6. Buffers

- 11662988122, Procell, 6 x 380 mL system buffer.
- 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution.
- 11930346122, Elecsys SysWash, 1 x 500 mL wash water additive.
- 11933159001, Adapter for SysClean.
- 11298500160, Elecsys SysClean.

8. WARNINGS, PRECAUTIONS AND NOTES

- Ensure the samples, calibrators and controls are at RT prior to measurement.
- Roche calibrators and controls should be analyzed/measured within 2 hours of reaching RT.
- For all reagents provided by Roche: Elecsys Anti-Sars-CoV-2 assay kit, PreciControl Anti-SARS-CoV-2 Negative, and Positive Control, follow vendor mandated expiration dates.
- Specimens should not be altered with additives (eg, biocides, antioxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.
- Do not use samples and controls stabilized with azide.

8.1. Sample Entry in VRD LabWare LIMS and Batch Creation

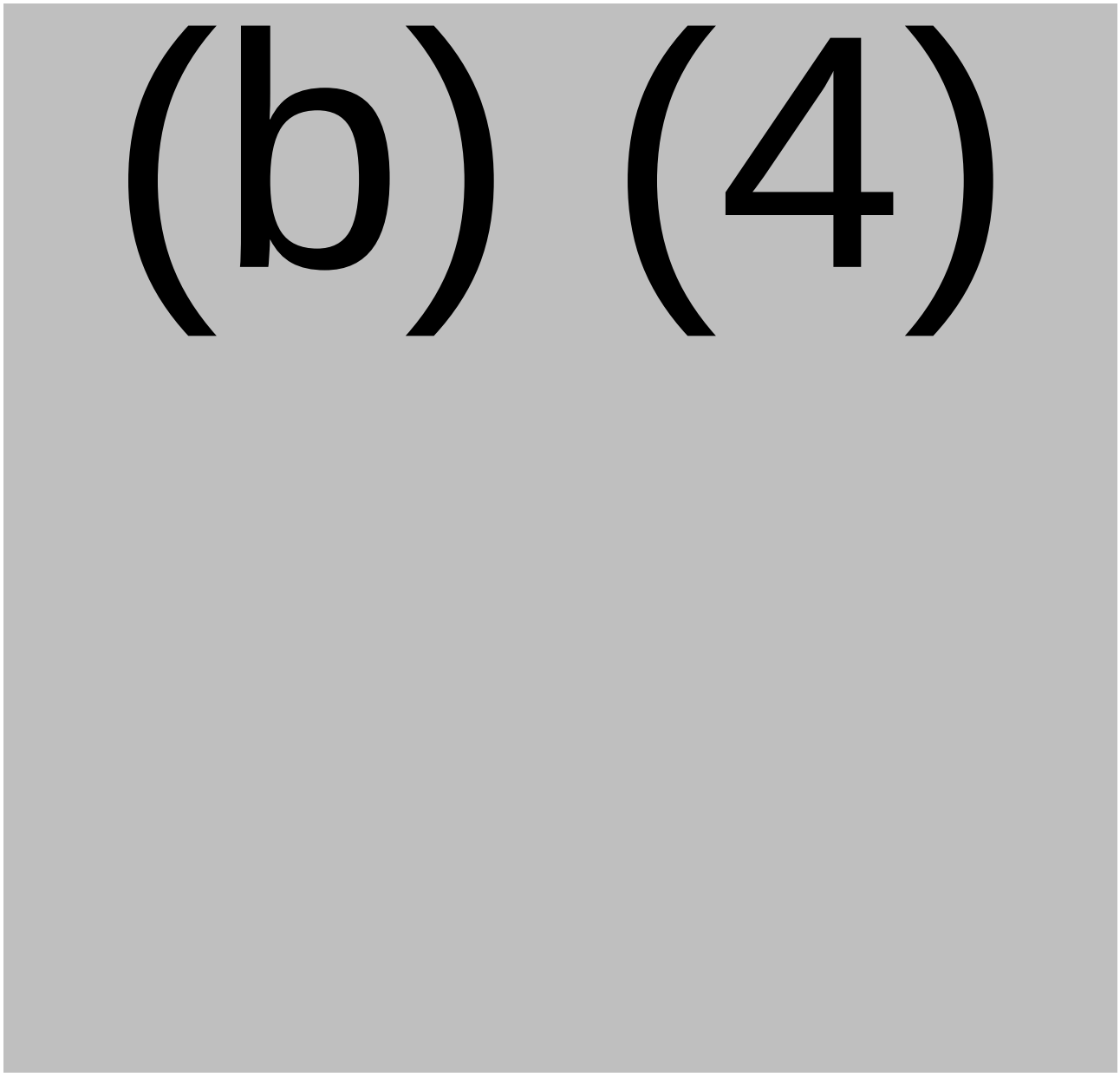
1. Obtain serum samples (in a workbox) to be processed from SM. Log in to LabWare LIMS and update workbox location when applicable (ie, lab freezer, benchtop).
2. If LIMS is unavailable, print out form VR-TM-10304-FM01 and VR-SOP-LC-10601-FM07 (Chain of Custody) to record all appropriate information during the sample processing steps.

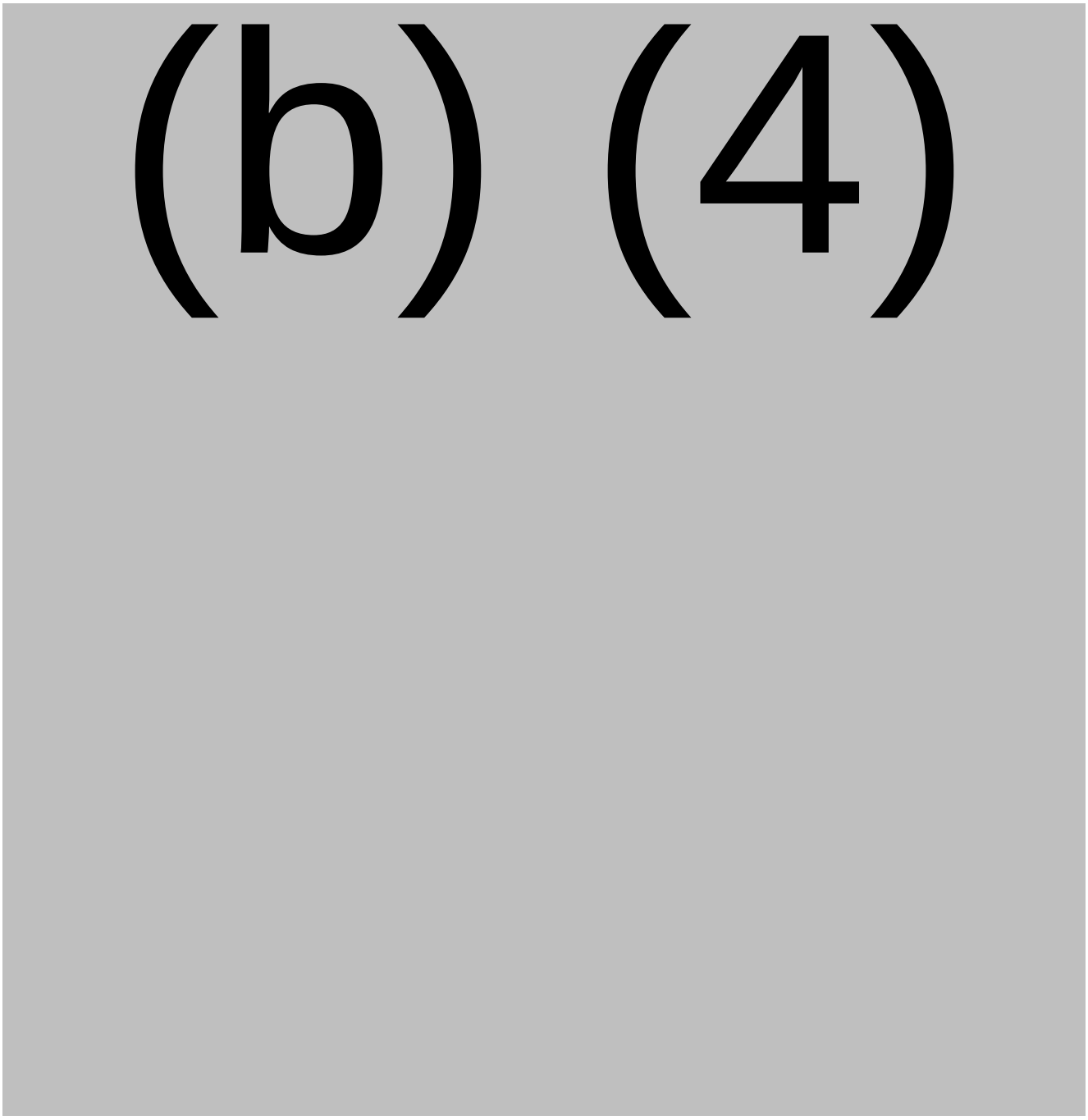
9. PROCEDURE

9.1. Obtaining Samples and Sample Entry in VRD LabWare LIMS

If LIMS is unavailable, print out form VR-TM-10304-FM01 and VR-SOP-LC-10601-FM07 (Chain of Custody) to record all appropriate information during the sample processing steps.

1. Remove sample workbox from the freezer and log workbox movement in LIMS using the following steps.





11. Thaw samples in workbox using the thawing station.

12. Once completely thawed, place workbox on (b) (4) at 25 RPM for approximately 5 minutes to mix the samples.

13. Centrifuge entire workbox for 2 minutes at 3000 x g, max acceleration, max deceleration.

10. STARTUP PROCEDURE

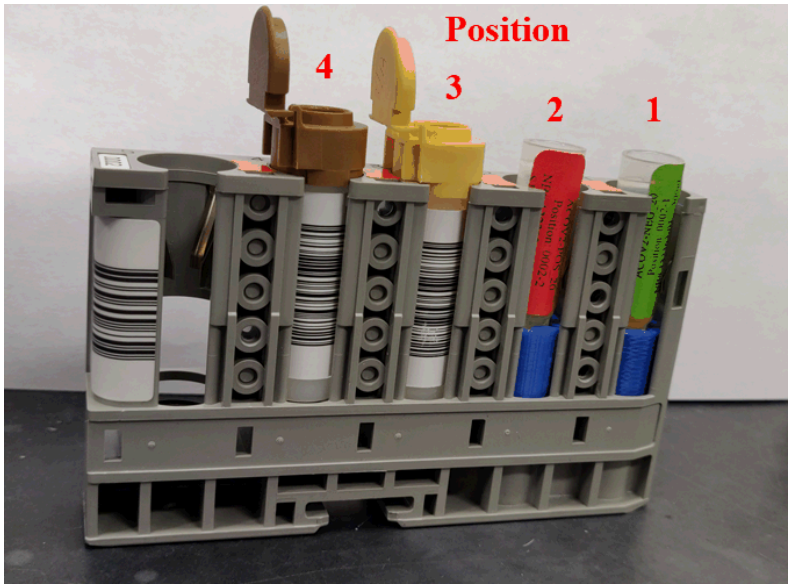
Refer to VR-SOP-FE-11297, Operation and Maintenance of the Roche cobas (b) (4) analyzer for the Startup Procedure.

10.1. Running QCs

1. Allow frozen QC vials to warm to RT before loading.

2. Once QC samples are thawed and at RT, place negative QC in position 1 and positive QC in position 2 of rack #0002. Place Roche PC ACOV2 1 in position 3 and Roche PC ACOV2 2 in position 4 of rack #0002 (Figure 4). Rack #0002 is designated as a QC rack in software.

Figure 4. QC Rack Example



3. Press **START** on right side of screen (Figure 5) and **START** on the **START** screen (Figure 6).

Figure 5. System Overview

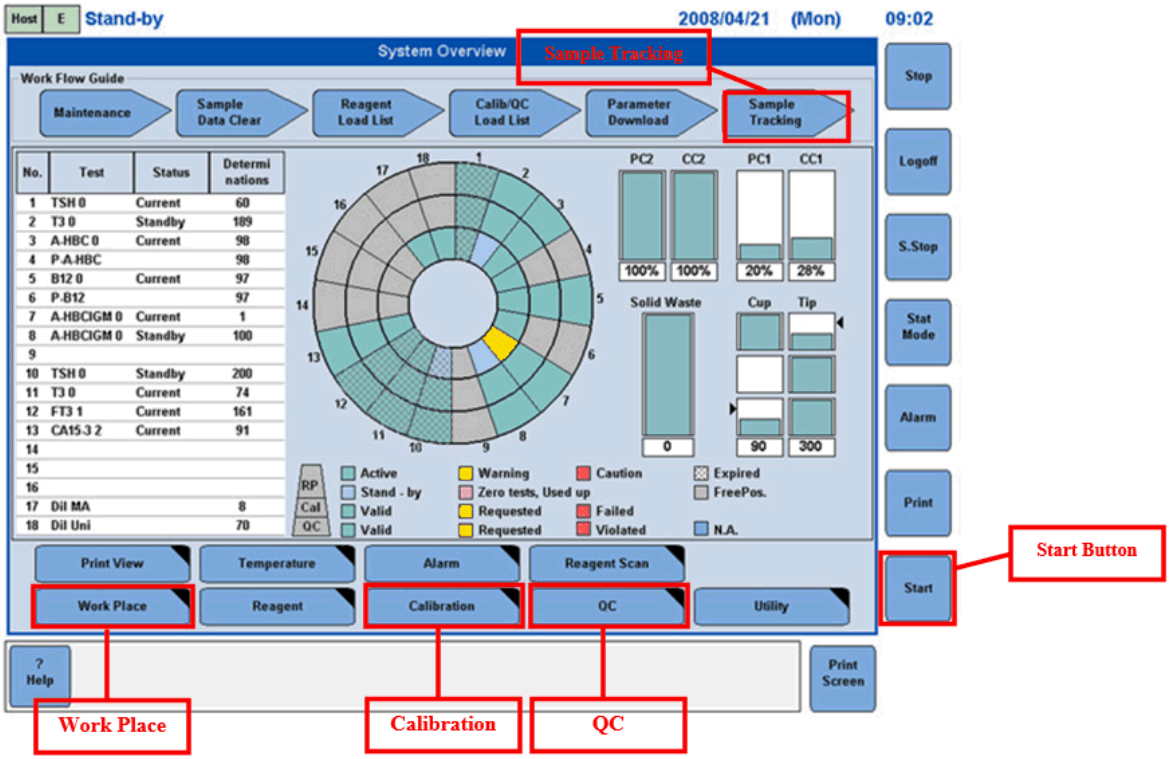
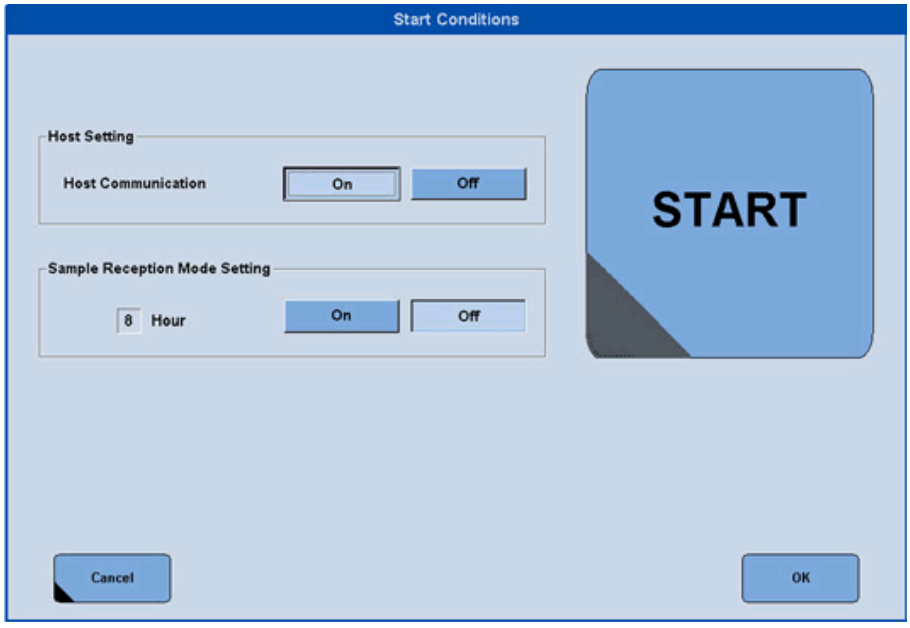
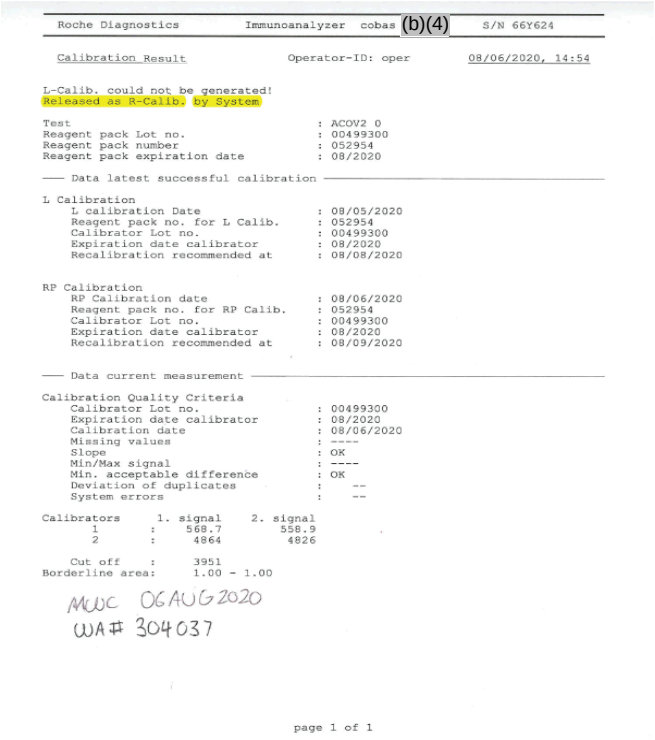


Figure 6. Start Conditions Window



4. Once the calibration and QCS are completed, the Roche cobas (b) (4) analyzer will print out a Calibration Form page (Figure 7) and a QCS Result page for each QCS.

Figure 7. Calibration Form Example Page



Roche Diagnostics Immunoanalyzer cobas (b)(4) S/N 66Y624

Calibration Result Operator-ID: oper 08/06/2020, 14:54

L-Calib. could not be generated!
Released as R-Calib. by System

Test	: ACOV2 0
Reagent pack Lot no.	: 00499300
Reagent pack number	: 052954
Reagent pack expiration date	: 08/2020

Data latest successful calibration

L Calibration

L calibration Date	: 08/05/2020
Reagent pack no. for L Calib.	: 052954
Calibrator Lot no.	: 00499300
Expiration date calibrator	: 08/2020
Recalibration recommended at	: 08/08/2020

RP Calibration

RP Calibration date	: 08/06/2020
Reagent pack no. for RP Calib.	: 052954
Calibrator Lot no.	: 00499300
Expiration date calibrator	: 08/2020
Recalibration recommended at	: 08/09/2020

Data current measurement

Calibration Quality Criteria

Calibrator Lot no.	: 00499300
Expiration date calibrator	: 08/2020
Calibration date	: 08/06/2020
Missing values	: ----
Slope	: OK
Min/Max signal	: ----
Min. acceptable difference	: OK
Deviation of duplicates	: --
System errors	: --

Calibrators	1. signal	2. signal
1	568.7	558.9
2	4864	4826

Cut off : 3951
Borderline area: 1.00 - 1.00

MCUC 06AUG2020
WA# 304037

page 1 of 1

5. **Confirm QCS results**: negative controls are “n-reac” and positive controls are “reac.”

6. On the Calibration Form page and QC result pages, write the Asset number, initials, and date (Figure 7).

7. Scan the Calibration Form and QCS Result pages as a pdf. Save the file name as (b)(4)

8. Save the Calibration Form pdf in the appropriate secure shared drive: ie, (b)(4)

10.2. Uploading into VRD LIMS LABWARE

(b)(4)

(b) (4)

10.2.1. Addition of Standby Reagent Packs, when Needed

1. Remove reagent pack from 4°C storage and allow to come to room temperature.

2. Open reagent pack turntable and place new reagent pack into an open slot. A maximum of 18 reagents packs can be placed in the turntable at one time.

3. Open the three reservoirs of the reagent pack and place the lids of each reservoir into a soft open position. The caps should cover the pack opening but not be pushed to a fully closed position.

4. Calibrate all new packs. To program a reagent calibration, go to the **Overview** screen. select **Calibration > Status**. Select all ACOV2 reagents listed in the table. Below **Method**, select **Full**, and select **Save**.

5. Check on **System Overview** to confirm that the calibration was assigned correctly.

6. To run QCS on a standby pack, go to **QC > Status > Standby bottle QC > check box > OK**. Check on **System Overview** (Figure 5) to see if processed correctly.

7. Place tube(s) in sample rack, barcodes facing out. Press **START**, and then **START** again (Figure 6).

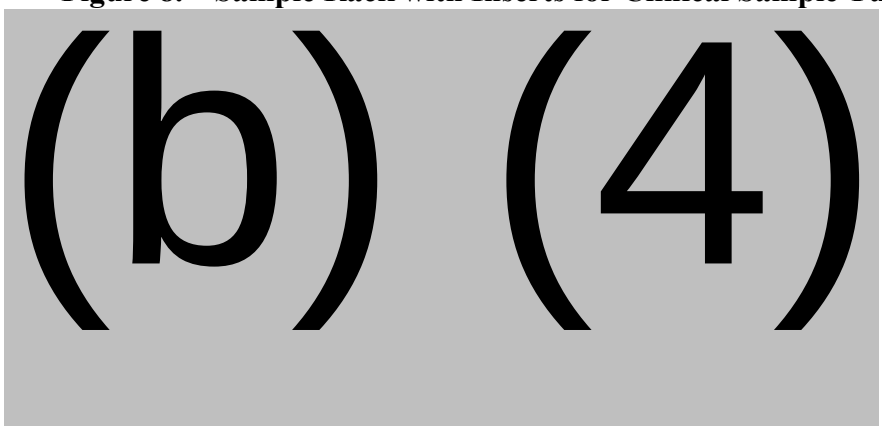
10.3. Testing of Patient Clinical Samples

10.3.1. Primary Method – Automated Barcode Scan

The analyzer can scan the tube barcodes prior to assigning the tests to each of the scanned barcodes.

1. Place barcoded samples into the rack (Figure 8) with the barcodes facing out. Do not use racks #0001 or #0002 for clinical samples. There are 5 sample positions per rack. Fifteen racks can be loaded onto the machine at one time. **Remove** all tube caps prior to loading the racks into the analyzer.

Figure 8. Sample Rack with Inserts for Clinical Sample Tubes

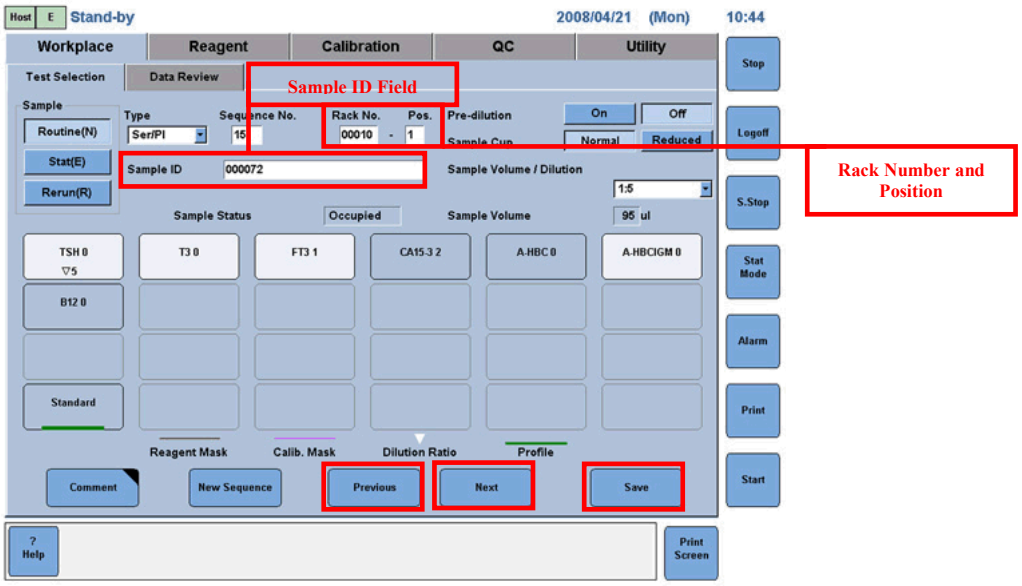


2. Load the racks into the loading tray and transfer the loading tray onto the A-Line track of the rack sampler.

3. From the **System Overview** screen (Figure 5), select the **Start** button. Rack and tube barcodes will be scanned by the analyzer. Once completed, the sample racks will be in the C-Line track of the rack sampler.

4. Select **Workplace > Test Selection** (Figure 9).

Figure 9. Test Selection Screen



5. Select **Previous** repeatedly until the rack, position, and barcode for the first sample is displayed in the Rack No, Pos, and Sample ID fields.

6. Select **ACOV2** to assign the SARS-CoV-2 N immunoassay testing to each sample barcode and **Save**.

7. Repeat Step 6 for all remaining samples.

8. Swap the loading trays between the C-Line track and A-Line track of the rack sampler. The sample racks do not need to be rearranged.

9. Select the **Start** button to begin the assay run (Figure 5) and **Start** on the **Start** screen (Figure 6).

If any sample barcodes were misread during the assay run, no results will be generated. Do NOT repeat. A repeat will be generated by the associated data reviewer.

IMPORTANT: Barcode(s) from any unprocessed samples must be recorded in a Batch Comment in LIMS.

10.3.1.1. Misread Sample Barcodes

If any sample barcodes were missed or incorrect, a sequence can be generated to add the sample barcode and test before starting the run.

1. From the **System Overview screen**, select the **Workplace tab** (Figure 5).

2. From the Workplace screen, select Test Selection.

3. Scan the sample barcode in the Sample ID field (Figure 9).

4. Select the **ACOV2** test under the **Sample Status** and select **Save**

5. Repeat steps 1-4 for all remaining samples.

6. Select the **Start** button (Figure 5) and **Start** on the **Start** screen (Figure 6) to begin the run.

If any sample barcodes were misread during the assay run, no results will be generated. Do NOT repeat. A repeat will be generated by the associated data reviewer.

IMPORTANT: Barcode(s) from any unprocessed samples must be recorded in a Batch Comment in LIMS.

10.3.2. Secondary Method – Assignment of Sample Testing Using the Handheld Barcode Scanner

The tubes can be placed within the racks prior to scanning. The positions of the tubes within the racks and the order of the racks is not important as the analyzer will cross match the scanned tube barcodes with the requested sample barcodes, as outlined below. If a tube barcode is scanned twice, it will only be tested once. If a tube barcode is not scanned, the tube will neither be tested nor flagged as an error during the barcode scanning.

1. Select **Workplace > Test Selection** (Figure 9).
2. Select **New Sequence** (ensuring **Routine(N)** is selected).
3. Select **Sample ID** field and scan the tube barcode using the handheld barcode scanner.
4. Select **ACOV2** to assign the SARS-CoV-2 N immunoassay testing.
5. Select **Save**.
6. Repeat steps 3-5 for all remaining samples.
7. Load the racks into the loading tray and transfer the loading tray to the A-Line of the rack sampler. **Remove** all tube caps prior to loading the racks into the analyzer.
8. Select the **Start** button (Figure 5) and **Start** on the **Start** screen (Figure 6) to begin the run.

If any sample barcodes were misread during the assay run, no results will be generated. Do NOT repeat. A repeat will be generated by the associated data reviewer.

IMPORTANT: Barcode(s) from any unprocessed samples must be recorded in a Batch Comment in LIMS.

11. DATA EXPORT

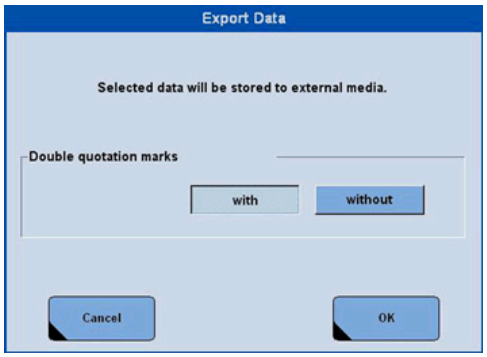
11.1. Data Export

Data can be transferred either through a USB flash drive or a USB Sharing device. The data can only be exported when the analyzer is in **Stand-by** mode. If the system is not in **Operation**, touch the global **Stop** button. Please note that selecting **Stop** during sample testing will abort the run.

If using a USB Sharing Device, ensure that the USB Sharing Device light indicator is set to Roche.

1. Once the analyzer is in **Stand-by** mode, touch **System Overview > Workplace > Data Review**.
2. Select all the test results to export. Select **Export Data**. Ensure the **With** button is selected for **Double quotation marks** in the **Export Data** window (Figure 10).

Figure 10. Data Export Window



3. As the data is being exported, the **Stand-by** mode will change to **Media Write** and return to **Stand-by** once completed. Never remove the USB key from the analyzer, unless it is in the **Stand-by** mode as this can damage the unit.

4. Select the **Remove USB** global button. Select **OK** when the confirmation window displays **Do you want to remove the USB drive?**

If the Roche cobas (b) (4) analyzer is connected to a USB device, proceed to Section 11.2.
If the Roche cobas (b) (4) analyzer is connected to a USB Sharing Device, proceed to Section 11.3.

11.2. Transfer using a USB Flash Drive

1. Once the **Please remove the USB device from the system** message appears, remove the USB flash drive from the USB port at the left side of the analyzer.

2. Insert the USB device onto the Roche cobas (b) (4) associated laptop.

3. Log onto the Roche cobas (b) (4) associated laptop and navigate to the USB drive (b) (4) csv's folder.

4. Ensure the correct number of data entries are present on the csv file.

5. The file name is exported onto the USB device as backup-yyyy-mm-dd, where yyyy-mm-dd represents the date the data was exported. Transfer the data file into the (b) (4) folder.

6. Rename the file as the batch name, ie, (b) (4) .

7. Copy and paste the data file into the appropriate network drive.

11.3. Transfer through a USB Sharing Device

1. Once the **Please remove the USB device from the system** message appears, use the switch (Figure 12) to change the output of the USB device from Roche to Laptop (Figure 11).
2. Ensure the USB Sharing Switch Light Indicator is changed to Laptop (Figure 11).
3. Plug in the USB data transfer cable into the Roche cobas (b) (4) associated laptop.
4. Log onto the Roche cobas (b) (4) associated laptop and navigate to the USB drive (b) (4) csv's folder.
5. Ensure the correct number of data entries are present on the csv file.
6. The file name is exported onto the USB device as backup-yyyy-mm-dd, where yyyy-mm-dd represents the date the data was exported. Transfer the data file into the (b) (4) folder.
7. Rename the file as the batch name, ie, (b) (4).
8. Copy and paste the data file into the appropriate network drive.

Figure 11. USB Sharing Switch Light Indicator

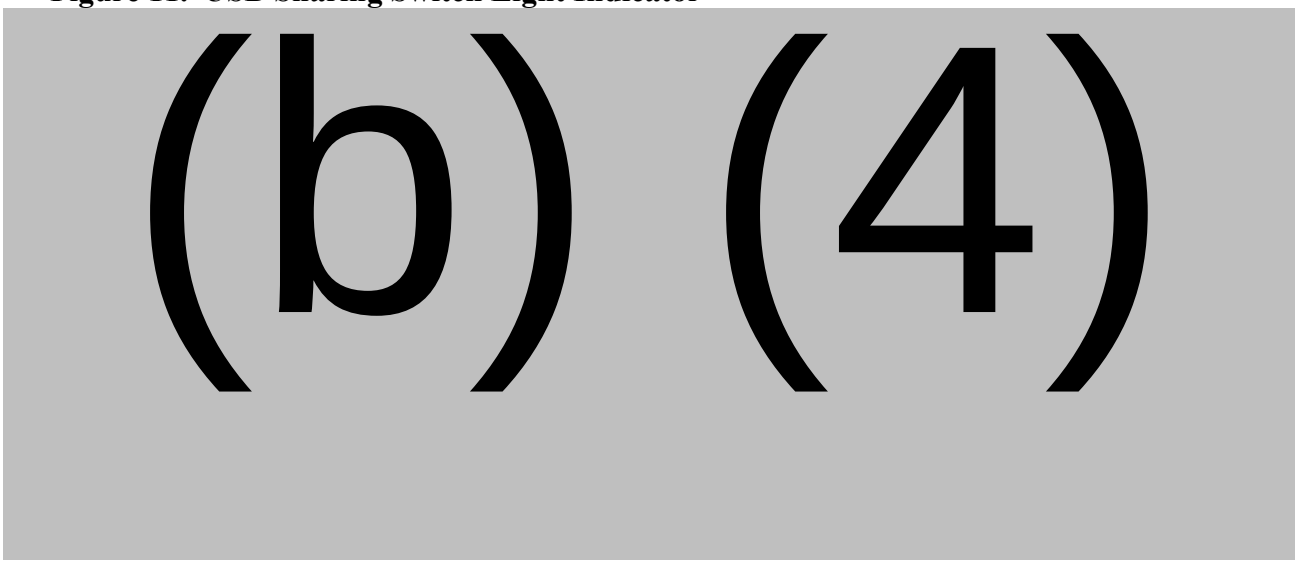
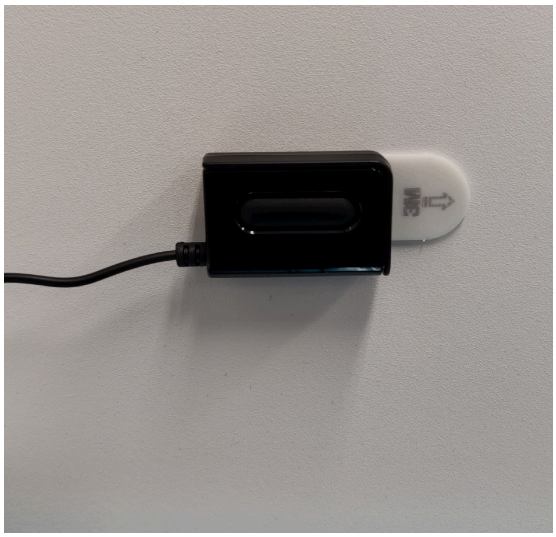


Figure 12. USB Sharing Switch

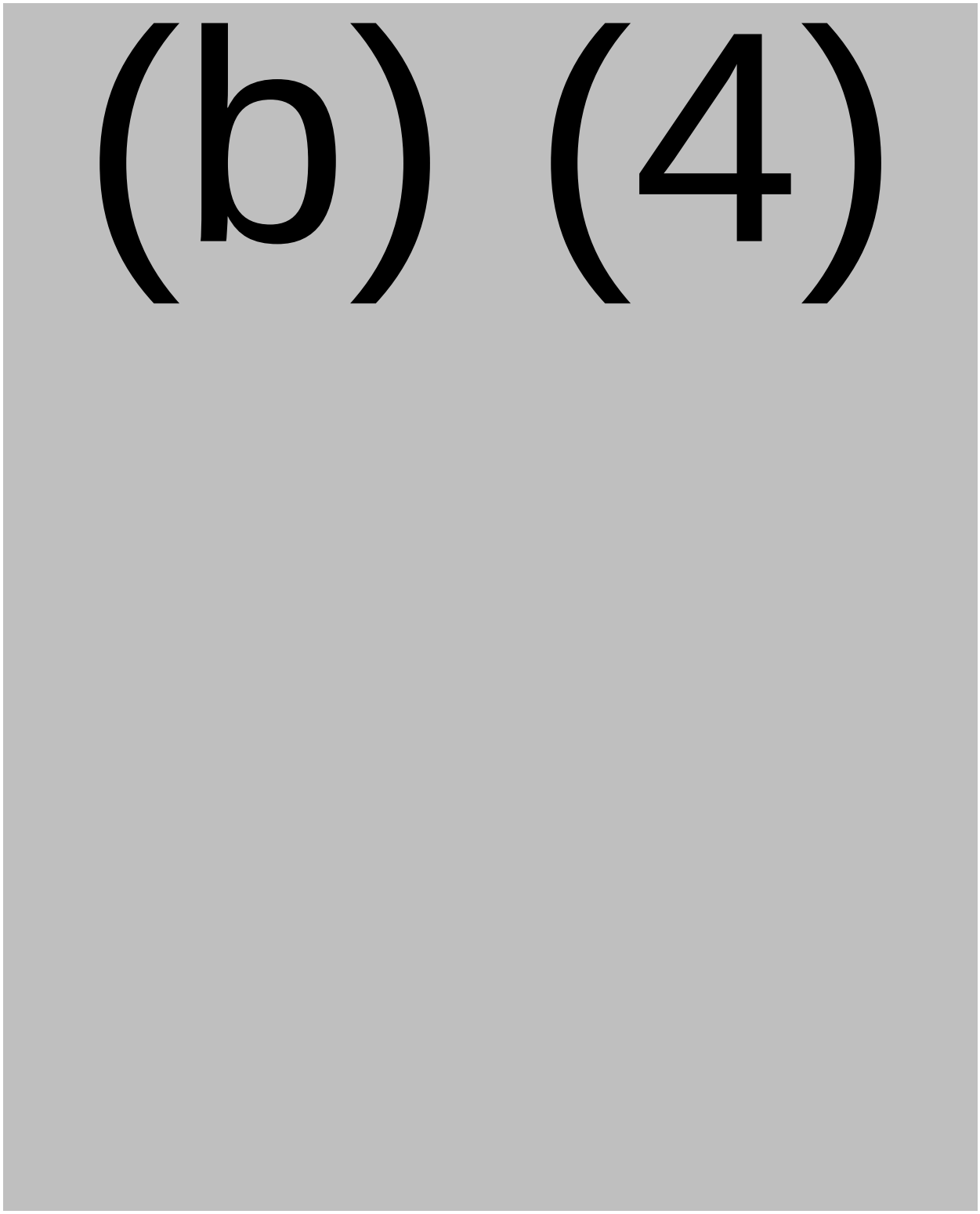


12. DOCUMENTATION

1. Raw data .csv files will be transferred to the secure network drive upon assay completion. Any other file types do not need to be transferred. Raw data is not required to be printed.

13. ASSAY METADATA COMPLETION

(b) (4)



14. REPEAT PROCESS

If a sample barcode is not successfully scanned by the Roche Cobas (b) (4) analyzer or an error occurs during the assay run, the sample will not be processed by the instrument. A repeat test is then generated for the misread sample barcode by the assay run's associated data reviewer.

When assigned a repeat batch, follow the steps below:

1. Remove workboxes associated with the repeat batch and track sample movement of workboxes from the freezer to the lab following Section 9.1.
2. Cherry-pick the repeat samples needed from each workbox.
3. Scan sample barcodes and assign tests following Section 10.3.1.1.
4. Enter rack and position of each sample (Figure 9).
5. Once all samples have been entered, select the **Start** button (Figure 5) and **Start** on the **Start** screen (Figure 6) to begin the run.
6. Once the samples complete processing, place the samples into their original workbox.
7. Enter all assay metadata following Section 13.
8. Track movement of samples back into the freezer following Section 13.

15. SHUTDOWN PROCEDURE

Please refer to VR-SOP-FE-11297, Operation and Maintenance of Roche cobas (b) (4) analyzer for the Shutdown procedure. Replace the USB device into the analyzer

16. INTERPRETATION OF RESULT

A qualified data reviewer analyzes the data according to pre-specified acceptance criteria, as described in VR-SOP-LC-11296.

17. REFERENCES

Table 5. General References

Document	Title
VR-SOP-LC-10586	Liquid Handling Techniques for Regulated Laboratory Procedures.
VR-SOP-LC-10601	Data Package Chain of Custody.
VR-SOP-QU-10004	Documentation Practices in Vaccine Research.
VR-SOP-LC-10774	Specimen Management Record Using Labware LIMS.
VR-SOP-QU-10726	Laboratory Deviation Report

Table 6. Form References

Form	Title
VR-TM-10304-FM01	Assay Completion Worksheet for the SARS CoV-2 Nucleocapsid (N) antigen Detection Assay
VR-SOP-LC-10601-FM07	Generic Assay Data Package Chain of Custody

Table 7. Specific References

Document	Title
VR-SOP-LC-11296	Data Review for SARS CoV-2 Nucleocapsid Antigen Detection Assay
VR-SOP-FE-11297	Operation of the Roche cobas(b) (4) Immunoassay Analyzer

18. DOCUMENT VERSION MODIFICATIONS

18.1. CRIF Number: VR-CRIF-20-14753

Table 8. Detailed Changes

List detailed changes for document(s) Include Section number(s) for each	List rationale for each change
Added Section 11.2 and Section 11.3.	To reflect current practices.
Updated Section 8 to include guidance for expiry date for reagents.	Clarity.
Updated Repeat Process for samples.	To reflect current practices.

Document Approval Record

Document Name:	VR-TM-10304
Document Title:	Test Method for the SARS CoV-2 Nucleocapsid (N) Antigen Detection Assay

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	23-Nov-2020 14:44:33	Final Approval
(b) (6)	24-Nov-2020 19:57:50	Author Approval
(b) (6)	24-Nov-2020 20:03:18	Quality Assurance Approval
(b) (4)	30-Nov-2020 13:47:46	Final Approval
(b) (6)	10-Dec-2020 14:52:21	Final Approval
(b) (6)	17-Dec-2020 20:15:32	Final Approval