BioPlex 2200 SARS-CoV-2 IgG Panel Training

BIO-RAD

Product Support, Clinical Immunology

January 2021

Agenda

- Clinical Overview
- SARS-CoV-2 IgG Panel and Assay Overview
- Results Interpretation and Information Flag
- Assay Performance
- Frequently Asked Questions
- Investigating Discrepant results
- Supporting Tools

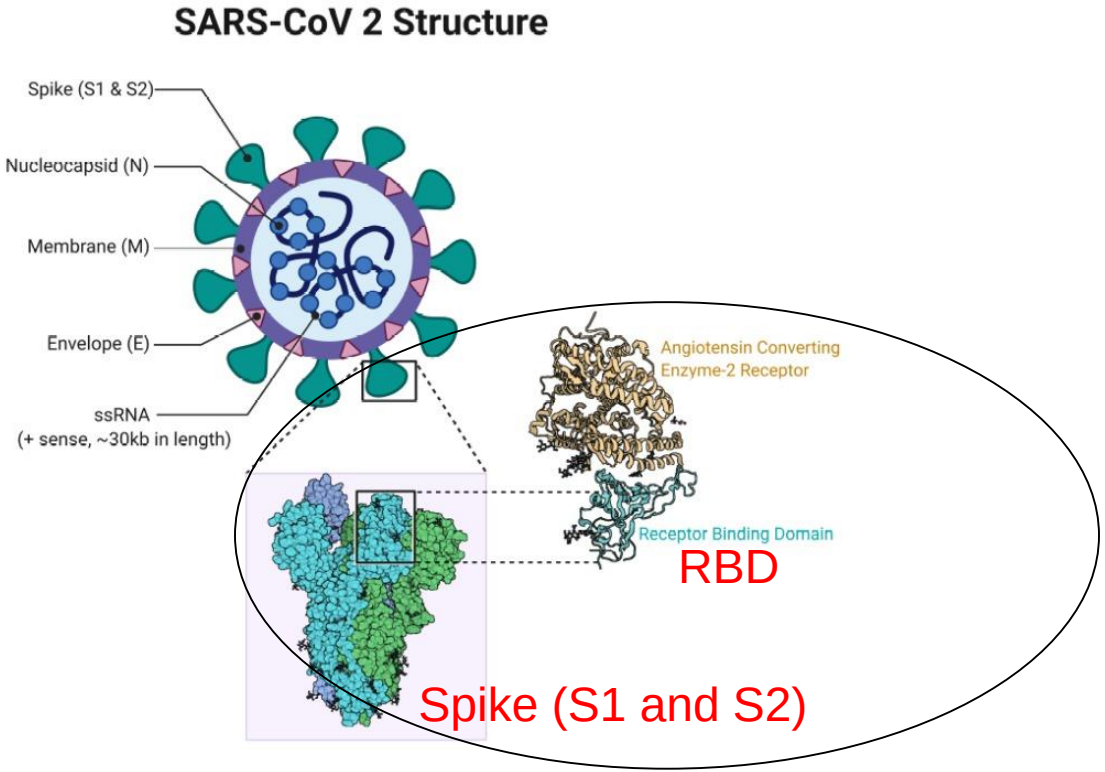
Terminology (1)

- SARS-CoV-2 (**S**evere **A**cute **R**espiratory **S**yndrome **C**orona**v**irus 2)
- COVID-19: disease caused by a novel coronavirus, SARS-CoV-2 (the pandemic)
- RT-PCR: (**R**everse **T**ranscription **P**olymerase **C**hain **R**eaction); the method to directly detect the virus and diagnose infection
- Immunity: the ability to resist a specific infection
- Vaccine: a biological preparation that induces immunity
- IgG: the antibody class that is a marker of immunity
- Antigen: foreign substance that induces an immune response

Terminology (2)

- S1: Spike protein subunit 1 (antigen)
- RBD: **R**eceptor-**B**inding **D**omain of the S1 subunit (antigen)
- S2: Spike protein subunit 2 (antigen)
- Nucleocapsid protein: protein inside the nucleocapsid attached to the viral RNA (antigen)
- Neutralizing antibody: antibody with the ability to stop the virus from infecting a cell most often with affinity to the S1 and RBD epitopes

SARS-CoV-2 Viral Structure



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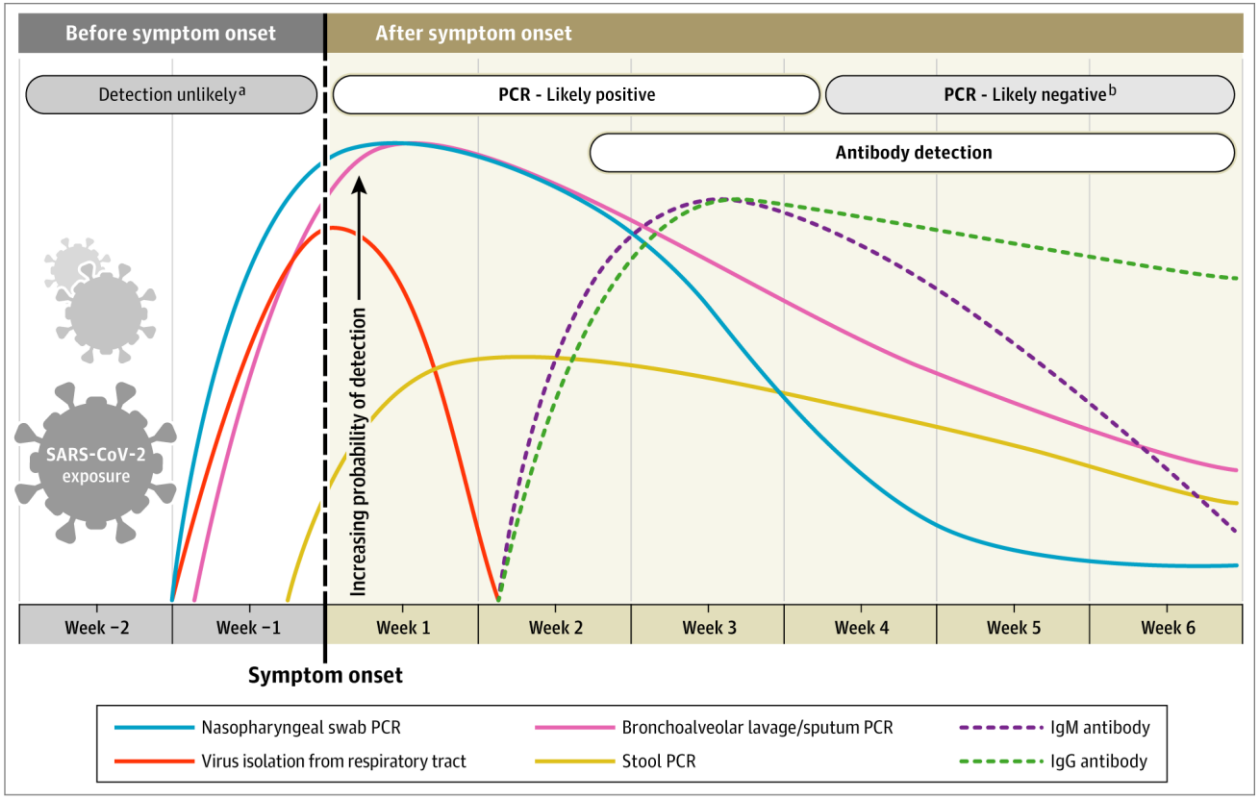
Laboratory Testing for SARS-CoV-2



JAMA June 9, 2020 Volume 323, Number 22, page 2250

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CDC Recommendations for Serological Testing

- Currently, there are **no guidelines** that indicate whether to test for IgA, IgM and IgG, or total antibody
- It is important to **minimize false positive test results** by choosing an assay with **high specificity** and by testing populations and individuals with an elevated likelihood of previous exposure to SARS-CoV-2.
- Antibodies most commonly become detectable 1-3 weeks after symptom onset, at which time suggests that infectiousness likely is great decreased and that some degree of immunity from future infection has developed.

Bio-Rad Laboratory Testing for SARS-CoV-2

Molecular Testing

Real Time PCR

- Bio-Rad CFX systems

Droplet Digital PCR

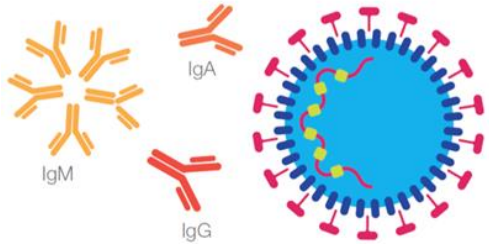
- Bio-Rad QX 200 system
- SARS-CoV-2 EUA Kit
- Exact Diagnostics SARS-CoV-2 Standard and Negative (QC)

Serology Testing

- SARS-CoV-2 Total Antibody EUA Test
- Evolis system
- Virotrol (Reactive) Viroclear (Non Reactive) QC

Real-Time PCR (RT-PCR)

Droplet Digital PCR (ddPCR)



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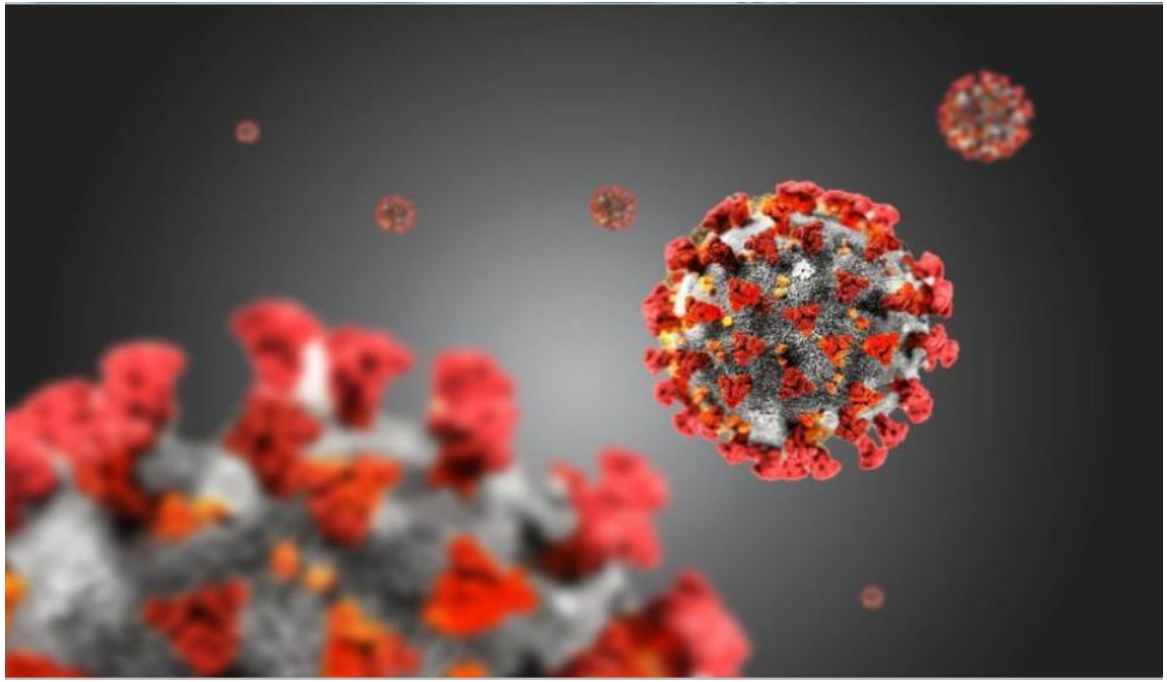
BioPlex 2200 SARS-CoV-2 IgG Panel

Proposed Intended Use (OUS-CE Mark)

The BioPlex 2200 SARS-CoV-2 IgG Panel is a multiplex assay for the qualitative detection and semi-quantitative differentiation of IgG class antibodies against the RBD, S1, S2, and nucleocapsid protein of the SARS-CoV-2 virus in human serum and plasma.

The BioPlex 2200 SARS-CoV-2 IgG Panel is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection or acquired immunity from vaccination to the virus.

BioPlex 2200 SARS-CoV-2 IgG Panel



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SARS-CoV-2 IgG Panel – Assay Overview

- Kit size: 200 tests per pack
  - IgG antibodies for RBD, S1, S2, Nucleocapsid markers
  - Qualitative screen
  - Semi-quantitative (U/mL) for individual marker results
  - Dynamic Range 1-100 U/mL

    * Cutoff is 10 U/mL

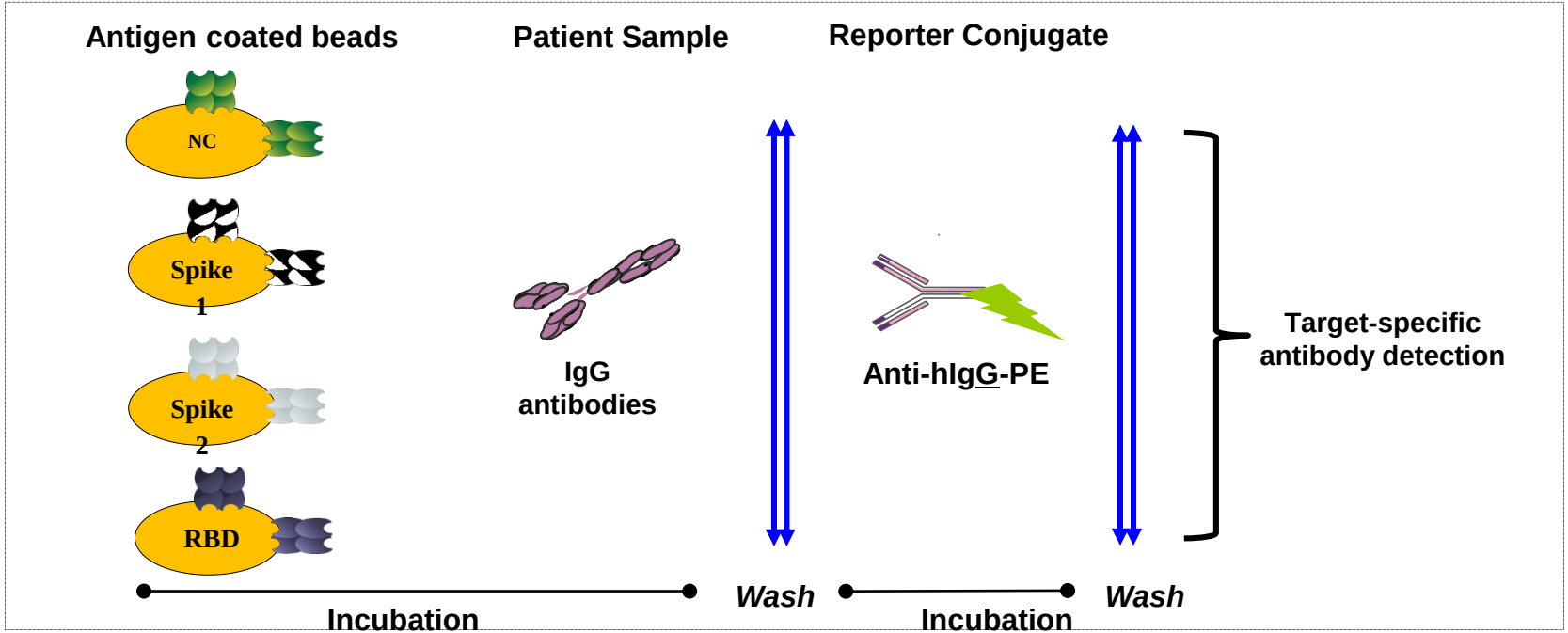
    Results > 100 U/mL : 1:8, 1:16 and 1:32 onboard dilutions
- Calibrator: 5 calibrator levels (in 6 vials) per set
  - 4PL calibration
- Control: 2 levels of control (in 3 vials)
  - 2 control sets per box
- SW 4.3 or later software with current hardware configuration

SARS-CoV-2 IgG Panel – Assay Overview

- Hardware configuration (recommended)
  -Stainless steel probes (sample and reagent)
  -High flow wash stations (sample and reagent)

BioPlex 2200 SARS-CoV-2 IgG Assay Design

Simultaneous detection with individual determination of Ab to SARS-CoV2 targets



QC beads: ISB and SVB

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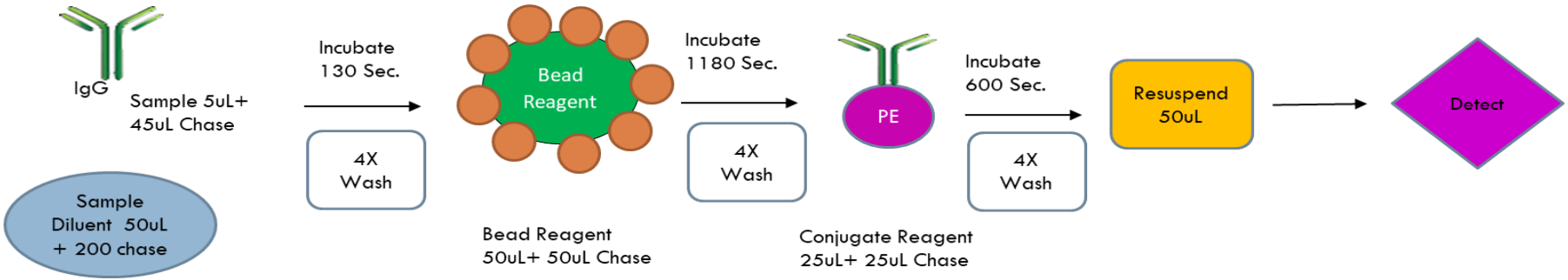
BioPlex 2200 SARS-CoV-2 IgG Assay Protocol



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SARS-CoV-2 IgG Reagent Pack

Assay Feature	SARS-CoV-2 IgG Pack
Catalog Number	12014192
Number of Tests	200
Intended Use	Qualitative & semi-quantitative detection of SARS-CoV-2 proteins in human serum or plasma
Beads	S1, S2, RBD, Nucleocapsid
Sample Type	Serum, Plasma
Sample Volume	5 uL (serum/plasma)
Time to First Results	45 minutes
Throughput	100 samples/hour
Type of Results	Qualitative screen plus Semi-quantitative
Reportable Range	0 – 100 U/mL
Interpretation	Negative (0 – 9 U/mL), Positive (10 – 100 U/mL)
Open Pack Stability	30 days at 2 – 8° C

Product Catalog Numbers

Catalog Number	Part Description
12014192	SARS-CoV-2 IgG Reagent Pack, 200 tests
12014193	SARS-CoV-2 IgG Calibrator Set
12014194	SARS-CoV-2 IgG Calibrator CD
12014195	SARS-CoV-2 IgG Control Set
12014196	SARS-CoV-2 IgG Control CD

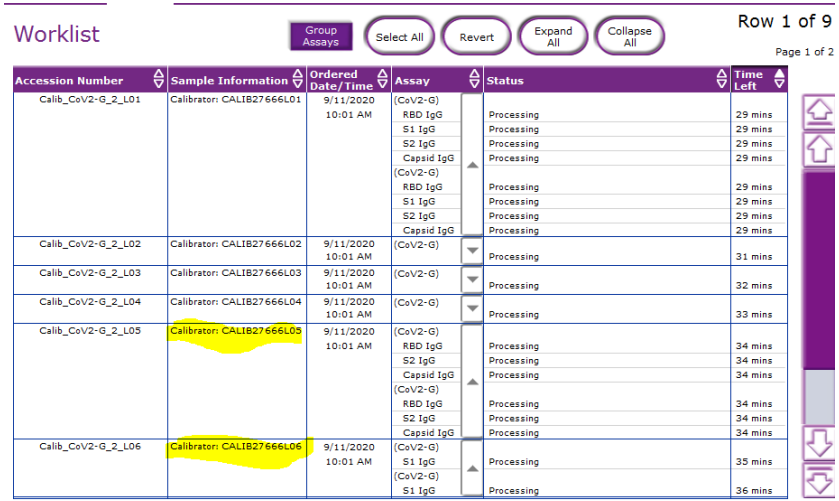
Additional Part Numbers

Catalog Number	Part Description
12014232	SARS-CoV-2 IgG APF, IFU, and CD Bundle
12014234	SARS-CoV-2 IgG APF CD, SW
665-0569A	SARS-CoV-2 IgG Reagent Pack IFU

SARS-CoV-2 IgG Calibrator & Control Set

Assay Feature	Calibrator	Control
Catalog Number	12014193	12014195
CD Catalog No.	12014194	12014196
Components	6 vials 5 positive, 1 negative	3 vials (2 sets) 2 positive, 1 negative
Calibration Curve Stability	30 days	NA
Open Vial Stability	60 days	60 days

Worklist –CALIBRATORS and CONTROLS



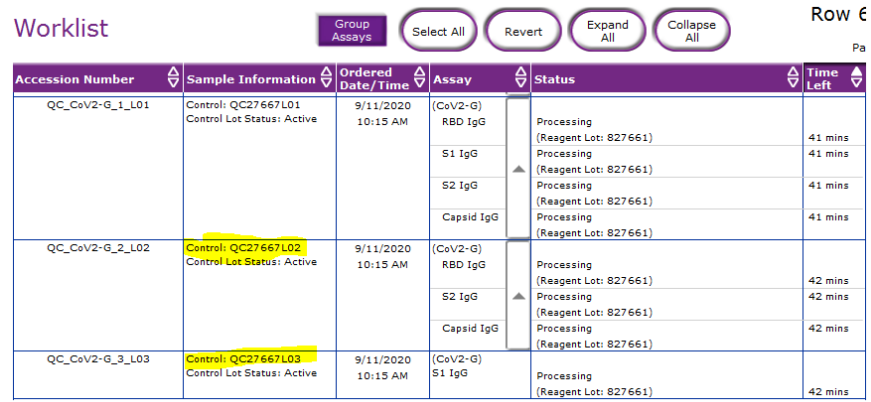
Worklist

Group Assays | Select All | Revert | Expand All | Collapse All

Row 1 of 9

Page 1 of 2

Accession Number	Sample Information	Ordered Date/Time	Assay	Status	Time Left
Calib_CoV2-G_2_L01	Calibrator: CALIB27666L01	9/11/2020 10:01 AM	(CoV2-G)		
			RBD IgG	Processing	29 mins
			S1 IgG	Processing	29 mins
			S2 IgG	Processing	29 mins
			Capsid IgG	Processing	29 mins
			(CoV2-G)		
			RBD IgG	Processing	29 mins
			S1 IgG	Processing	29 mins
			S2 IgG	Processing	29 mins
			Capsid IgG	Processing	29 mins
Calib_CoV2-G_2_L02	Calibrator: CALIB27666L02	9/11/2020 10:01 AM	(CoV2-G)	Processing	31 mins
Calib_CoV2-G_2_L03	Calibrator: CALIB27666L03	9/11/2020 10:01 AM	(CoV2-G)	Processing	32 mins
Calib_CoV2-G_2_L04	Calibrator: CALIB27666L04	9/11/2020 10:01 AM	(CoV2-G)	Processing	33 mins
Calib_CoV2-G_2_L05	Calibrator: CALIB27666L05	9/11/2020 10:01 AM	(CoV2-G)		
			RBD IgG	Processing	34 mins
			S2 IgG	Processing	34 mins
			Capsid IgG	Processing	34 mins
			(CoV2-G)		
			RBD IgG	Processing	34 mins
			S2 IgG	Processing	34 mins
			Capsid IgG	Processing	34 mins
Calib_CoV2-G_2_L06	Calibrator: CALIB27666L06	9/11/2020 10:01 AM	(CoV2-G)		
			S1 IgG	Processing	35 mins
			(CoV2-G)		
			S1 IgG	Processing	36 mins



Worklist

Group Assays | Select All | Revert | Expand All | Collapse All

Row 6

Pa

Accession Number	Sample Information	Ordered Date/Time	Assay	Status	Time Left
QC_CoV2-G_1_L01	Control: QC27667L01 Control Lot Status: Active	9/11/2020 10:15 AM	(CoV2-G)		
			RBD IgG	Processing (Reagent Lot: 827661)	41 mins
			S1 IgG	Processing (Reagent Lot: 827661)	41 mins
			S2 IgG	Processing (Reagent Lot: 827661)	41 mins
			Capsid IgG	Processing (Reagent Lot: 827661)	41 mins
QC_CoV2-G_2_L02	Control: QC27667L02 Control Lot Status: Active	9/11/2020 10:15 AM	(CoV2-G)		
			RBD IgG	Processing (Reagent Lot: 827661)	42 mins
			S2 IgG	Processing (Reagent Lot: 827661)	42 mins
			Capsid IgG	Processing (Reagent Lot: 827661)	42 mins
QC_CoV2-G_3_L03	Control: QC27667L03 Control Lot Status: Active	9/11/2020 10:15 AM	(CoV2-G) S1 IgG	Processing (Reagent Lot: 827661)	42 mins

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SARS CoV-2 IgG Calibrator & Control Set

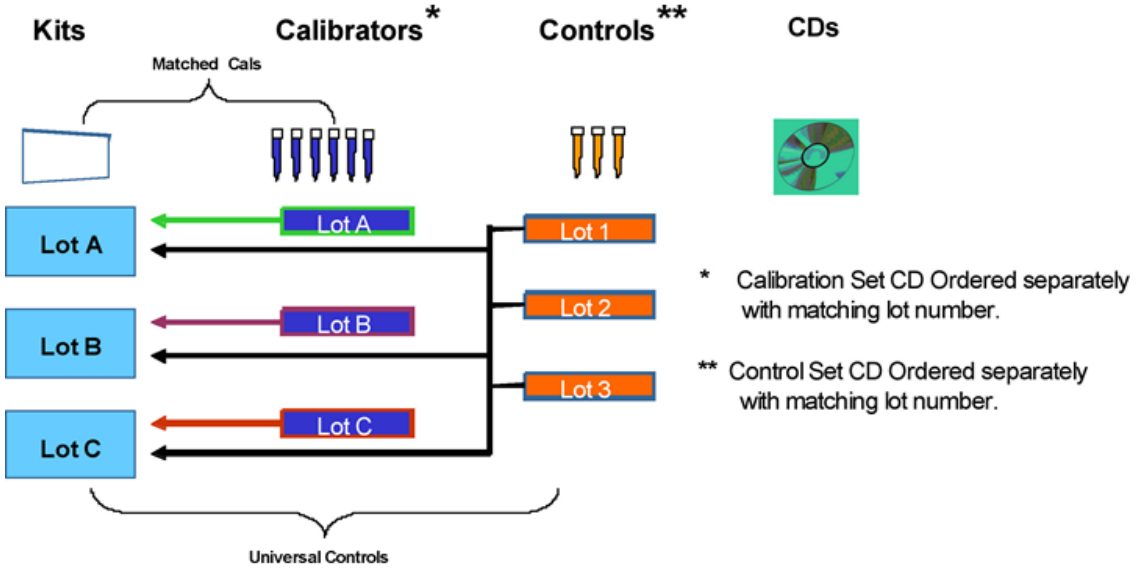
Calibrator and Control Handling:

- Allow the vials to reach room temperature (18º – 25ºC) and gently mix by inversion to ensure homogeneity.
- After each use, promptly replace the cap and return to 2º – 8ºC storage.

Quality Control must be performed:

- After assay calibration.
- Once per day testing is performed and with each new reagent pack lot.

Kit, Calibrator, and Control Usage



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Specimen Collection & Handling

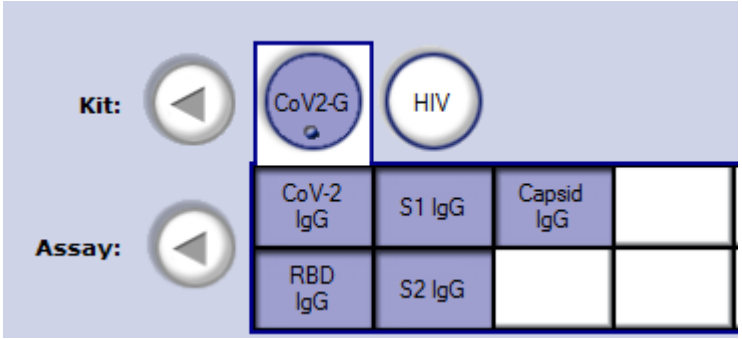
- Specimen Type
  - Serum
  - Plasma (K2 or K3 EDTA, Lithium or Sodium Heparin, Sodium Citrate)

- Specimen Storage
  - Up to five (5) days at room temp.
  - Up to seven (7) days at 2 – 8^{o} C
  - -20^{o} C for longer storage (at least 10 months)

- Specimen Preparation
  - Thoroughly mix thawed specimens
  - Up to five (5) freeze/thaw cycles acceptable

Test Order and Results Output

- “CoV2-G” Kit
- The customer orders a “CoV-2 IgG” (Qualitative)
    - “Negative” (all markers are negative)
    - “Positive” (at least one marker is positive)
- Customer orders all markers (Semi –Quantitative)
    - “RBD IgG” (U/mL)
    - “S1 IgG” (U/mL)
    - “S2 IgG” (U/mL)
    - “Capsid IgG” (U/mL)



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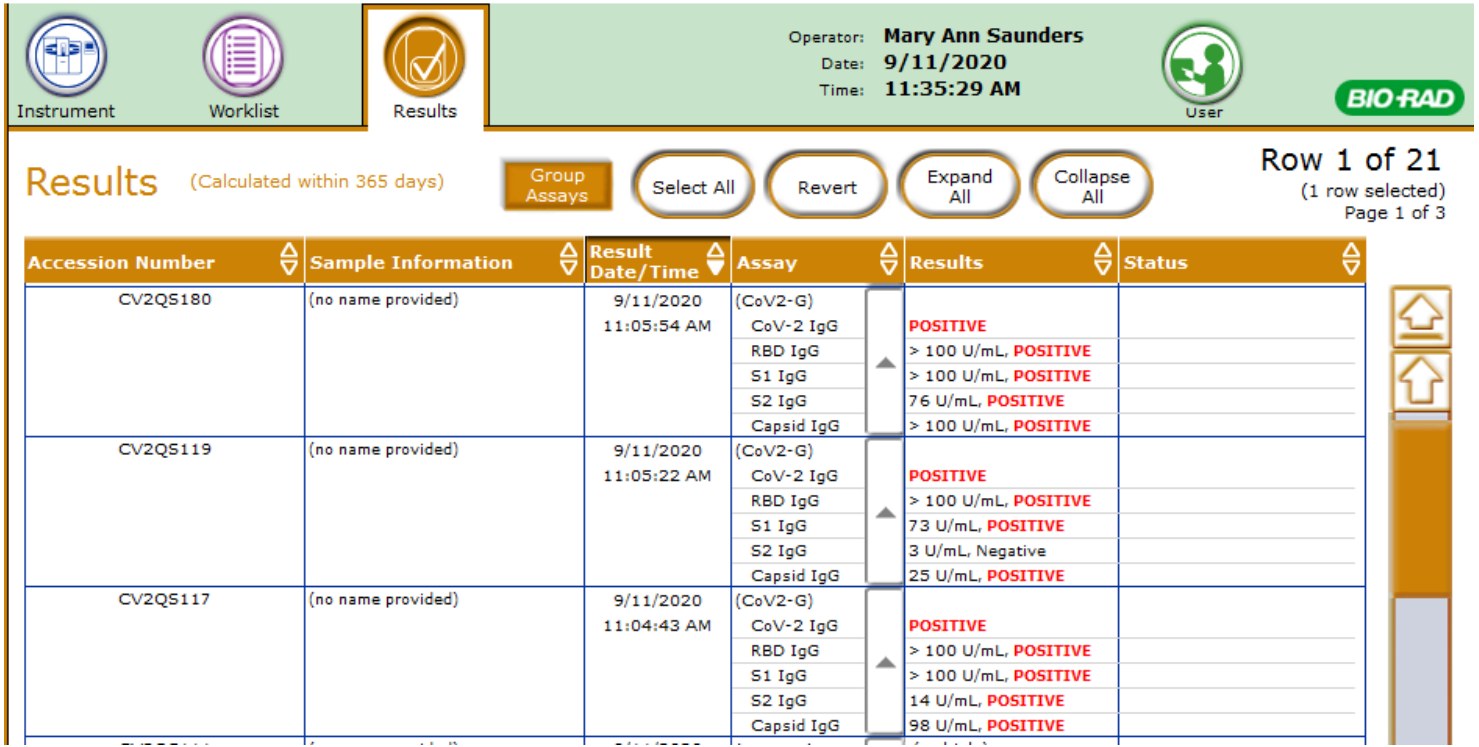
Results Output



Accession Number	Sample Information	Result Date/Time	Assay	Results	Status
CV2QS180	(no name provided)	9/11/2020 11:05:54 AM	(CoV2-G)		
			CoV-2 IgG	POSITIVE	
			RBD IgG	> 100 U/mL, POSITIVE	
			S1 IgG	> 100 U/mL, POSITIVE	
			S2 IgG	76 U/mL, POSITIVE	
			Capsid IgG	> 100 U/mL, POSITIVE	
CV2QS119	(no name provided)	9/11/2020 11:05:22 AM	(CoV2-G)		
			CoV-2 IgG	POSITIVE	
			RBD IgG	> 100 U/mL, POSITIVE	
			S1 IgG	73 U/mL, POSITIVE	
			S2 IgG	3 U/mL, Negative	
			Capsid IgG	25 U/mL, POSITIVE	
CV2QS117	(no name provided)	9/11/2020 11:04:43 AM	(CoV2-G)		
			CoV-2 IgG	POSITIVE	
			RBD IgG	> 100 U/mL, POSITIVE	
			S1 IgG	> 100 U/mL, POSITIVE	
			S2 IgG	14 U/mL, POSITIVE	
			Capsid IgG	98 U/mL, POSITIVE	

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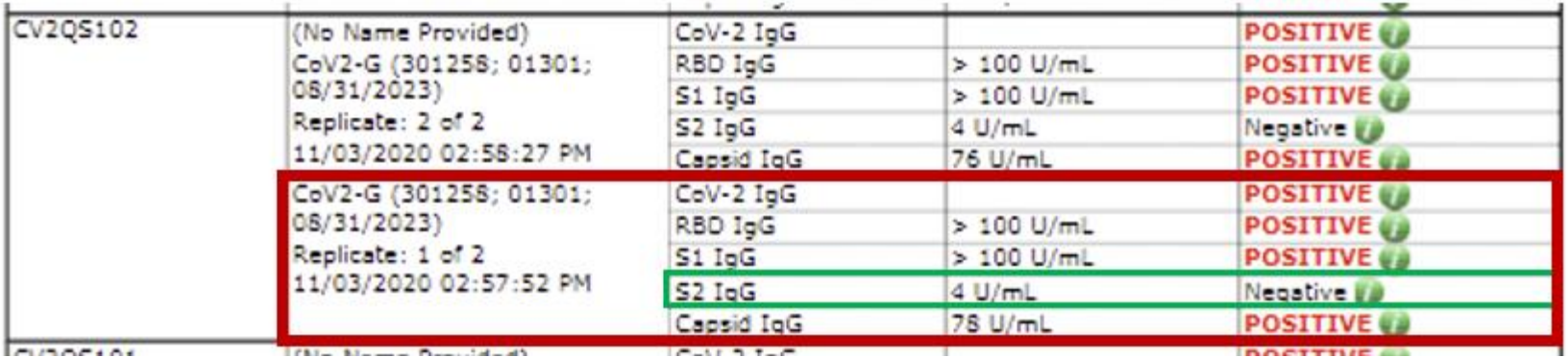
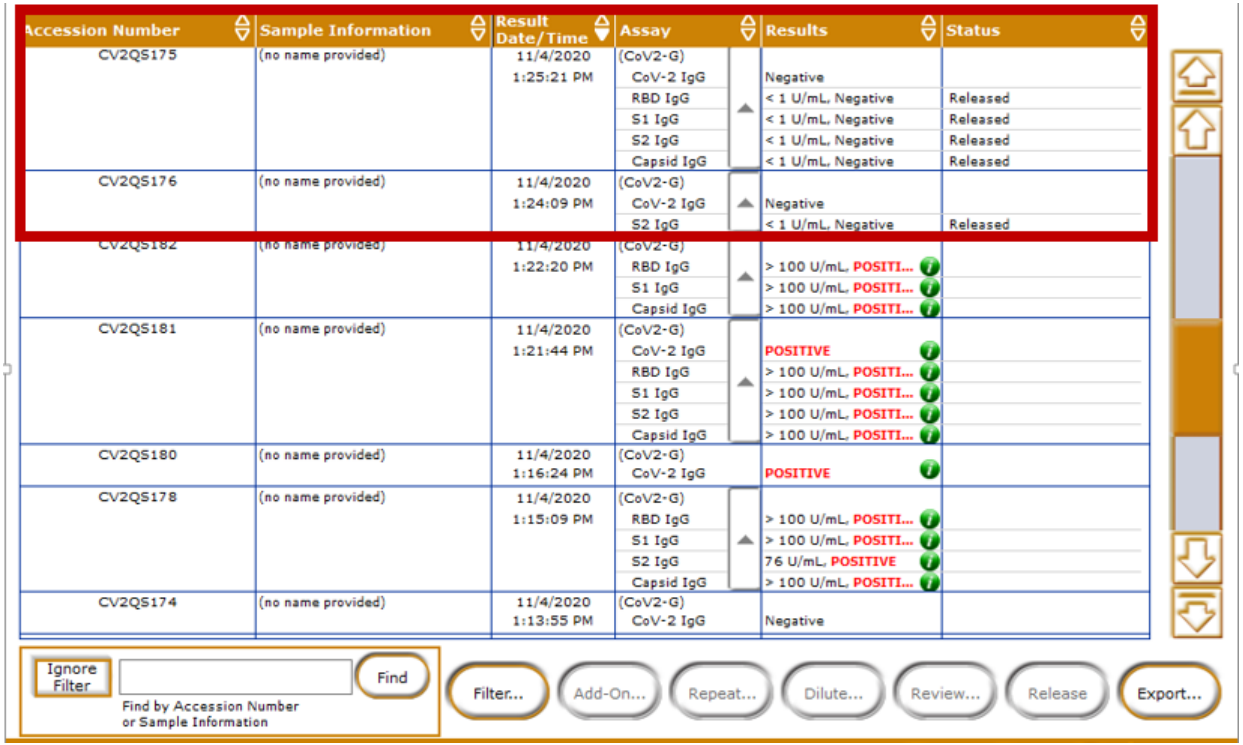
Information Flag

- Flag Number: -100008 Information (green icon)
- Description: One or more analyte is POSITIVE



CV2QS102	(No Name Provided)	CoV-2 IgG		POSITIVE
	CoV2-G (301258; 01301; 06/31/2023)	RBD IgG	> 100 U/mL	POSITIVE
		S1 IgG	> 100 U/mL	POSITIVE
	Replicate: 2 of 2	S2 IgG	4 U/mL	Negative
	11/03/2020 02:58:27 PM	Capsid IgG	76 U/mL	POSITIVE
	CoV2-G (301258; 01301; 06/31/2023)	CoV-2 IgG		POSITIVE
		RBD IgG	> 100 U/mL	POSITIVE
	Replicate: 1 of 2	S1 IgG	> 100 U/mL	POSITIVE
	11/03/2020 02:57:52 PM	S2 IgG	4 U/mL	Negative
		Capsid IgG	78 U/mL	POSITIVE

Information Flag



Accession Number	Sample Information	Result Date/Time	Assay	Results	Status
CV2QS175	(no name provided)	11/4/2020 1:25:21 PM	(CoV2-G)		
			CoV-2 IgG	Negative	
			RBD IgG	< 1 U/mL, Negative	Released
			S1 IgG	< 1 U/mL, Negative	Released
			S2 IgG	< 1 U/mL, Negative	Released
			Capsid IgG	< 1 U/mL, Negative	Released
CV2QS176	(no name provided)	11/4/2020 1:24:09 PM	(CoV2-G)		
			CoV-2 IgG	Negative	
			S2 IgG	< 1 U/mL, Negative	Released
CV2QS182	(no name provided)	11/4/2020 1:22:20 PM	(CoV2-G)		
			RBD IgG	> 100 U/mL, POSITI...	
			S1 IgG	> 100 U/mL, POSITI...	
			Capsid IgG	> 100 U/mL, POSITI...	
CV2QS181	(no name provided)	11/4/2020 1:21:44 PM	(CoV2-G)		
			CoV-2 IgG	POSITIVE	
			RBD IgG	> 100 U/mL, POSITI...	
			S1 IgG	> 100 U/mL, POSITI...	
			S2 IgG	> 100 U/mL, POSITI...	
			Capsid IgG	> 100 U/mL, POSITI...	
CV2QS180	(no name provided)	11/4/2020 1:16:24 PM	(CoV2-G)		
			CoV-2 IgG	POSITIVE	
CV2QS178	(no name provided)	11/4/2020 1:15:09 PM	(CoV2-G)		
			RBD IgG	> 100 U/mL, POSITI...	
			S1 IgG	> 100 U/mL, POSITI...	
			S2 IgG	76 U/mL, POSITIVE	
			Capsid IgG	> 100 U/mL, POSITI...	
CV2QS174	(no name provided)	11/4/2020 1:13:55 PM	(CoV2-G)		
			CoV-2 IgG	Negative	

Ignore Filter | Find | Find by Accession Number or Sample Information

Filter... | Add-On... | Repeat... | Dilute... | Review... | Release | Export...

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Information Flag – On Board Dilutions

CV2QS177	(no name provided)	11/4/2020 3:10:56 PM	(CoV2-G) RBD IgG	440 U/mL, POSITIVE	Released, Dilute, [O 1:32]
			S1 IgG	188 U/mL, POSITIVE	Released, Dilute, [O 1:32]
			Capsid IgG	232 U/mL, POSITIVE	Released, Dilute, [O 1:32]
		11/4/2020 3:09:10 PM	(CoV2-G) RBD IgG	531 U/mL, POSITIVE	Released, Dilute, [O 1:8]
			S1 IgG	206 U/mL, POSITIVE	Released, Dilute, [O 1:8]
			Capsid IgG	307 U/mL, POSITIVE	Released, Dilute, [O 1:8]
		11/4/2020 3:07:20 PM	(CoV2-G) CoV-2 IgG	POSITIVE	Repeat
		11/4/2020 1:14:32 PM	(CoV2-G) CoV-2 IgG	POSITIVE	Repeated
			RBD IgG	> 100 U/mL, POS...	Diluted
			S1 IgG	> 100 U/mL, POS...	Diluted
			S2 IgG	52 U/mL, POSITI...	
			Capsid IgG	> 100 U/mL, POS...	Diluted

- If a sample is diluted, a diluted sample icon and on board dilution information will appear.
- The green information flag is grayed out on the initial result (>100 U/mL)

Data Analysis

- Samples should initially be tested **undiluted**. If any individual antibody marker has a result of **>100 U/mL**, a test order should be placed for an **on-board dilution** of that specific marker to determine the antibody level.
- When performing on-board dilutions, report the result of the **lowest dilution** that has a result within the analytical range of the assay.
- The **overall result** from the initial SARSCoV-2 IgG Screen should be reported **in addition to a final result for individual analytes**, determined by the initial assay or subsequent on-board dilution.

Performance Summary (IFU)

- Specificity (pre-November 2019 samples)
  - 99.8% specificity in a healthy population (including pregnant women)
  - 100% specificity for human anti-mouse antibody (HAMA) samples
  - 99.8% specificity for infectious disease samples
  - 99.2% specificity for autoimmune disease samples

- Sensitivity
  - 96.3% sensitivity at ≥15 days post symptom onset

Preliminary data, based on development testing

Performance Summary (IFU)

- Serum vs plasma (K2 EDTA, K3 EDTA, Lithium Heparin, and Sodium Citrate)
  - No matrix effect for all targets
- Reproducibility- within run, between run, between day, between instrument/ site and total variation

Total %CV

-RBD IgG : < 9%

-S1 IgG: < 8%

-S2 IgG: < 6%

-Nucleocapsid: < 9%

Competitor Grid (FDA-EUA)

Company	Instrument	Ig Class	Target	Qual / Quant	Sample Type	Comments
Abbott	Alinity, Architect	IgG	Nucleocapsid	Qualitative	serum, plasma	Quest
Beckman Coulter	Access 2, DxI 600, DxI 800	IgG	RBD	Qualitative	serum, plasma	IgM in development
bioMérieux	Vidas	IgG, IgM	RBD	Qualitative	serum, plasma	
DiaSorin	LIAISON XL	IgG	S1 / S2	Qualitative	serum, plasma	
Diazyme	DZ-lite 3000 Plus	IgG	Spike and nucleocapsid	Qualitative	serum, plasma	IgM in development
Luminex	xMAP	IgG	S1, RBD, nucleocapsid	Qualitative	Serum, plasma	only other multiplex panel
Ortho	VITROS	Total Ig, IgG	Spike protein	Qualitative	serum	Quest
Roche	Elecsys	Total Ig	Nucleocapsid	Qualitative	Serum, plasma	LabCorp
Siemens	Atellica IM, ADVIA Centaur	Total Ig, IgG	RBD	Total Ig Qualitative, IgG Semi-quant	Serum, plasma	Only other semi-quantitative assay

*does not include microplate or lateral flow assays

Positioning: Multiplex

- RBD and S1
  - Detects past infections
  - Detect neutralizing antibodies
  - Most likely targets for vaccine development and vaccination monitoring
- S2
  - Unknown why a few patients only react to S2
- Nucleocapsid
  - Detect past infection

Positioning: Semi-Quantitation

- Measure antibody "titer" to screen for convalescent plasma
- Determination of dominant antibody response
- Measure antibody levels over time to determine length of immunity from infection
- Measure antibody levels over time to determine length of immunity from vaccine

Frequently Asked Questions

What is the benefit of 4 targets?

- It provides more information
  - Recognize different patient populations
  - Ability to perform orthogonal test algorithm in a single reaction
  - Possible differentiation of antibodies from infection versus antibodies from vaccination dependent on the vaccine target

What is “RBD” and why is it important?

- The receptor-binding domain or RBD is the epitope of the S1 that directly attaches to the ACE-2 receptor on the cell allowing the virus to infect the cell
- Antibodies that attach to the RBD can block the virus's ability to infect cells and “neutralize” the virus
- The RBD is the most likely target for vaccine development

What is S2 and why is it important?

- The S2 is part of the spike protein of the virus
- In our initial testing, we found a small subset of patients that only developed anti-S2 antibodies
- Having S2 increases the sensitivity (slightly) and may provide more clinical information in the future

Is the nucleocapsid protein target the same as in the Platelia kit?

- The Platelia kit uses an *E. coli* derived protein
- The BioPlex 2200 panel uses a CHO cell derived protein
- The two assays show high concordance

Why only IgG and not a total assay?

- IgG is more specific and longer lived
- IgG can be measured semi-quantitatively
- IgG is the primary antibody that provides immunity
- The total antibody assay can detect seroconversion in some patients 2-3 days sooner than the IgG assay after which there is no advantage to detecting total antibody

Investigating Discrepant Results

- What was the RT-PCR result?
- How many days of symptom onset?
- Who is the manufacturer of the other method?
  - What antibody isotype does it detect?
  - What antigenic marker does it use?
  - What is the assay format?
- What was the result on the other method?
  - Qualitative (positive/negative)
  - Quantitative (sample/cut off ratio)

Supporting Tools

Livelink

- **APF Data Files**

http://livelinkprd.bio-rad.com/livelink/livelink.exe?func=ll&objId=77314449&objAction=browse&viewType=1Assay Protocol File

- **Calibrator Data File**

http://livelinkprd.bio-rad.com/livelink/livelink.exe?func=ll&objId=77471825&objAction=browse

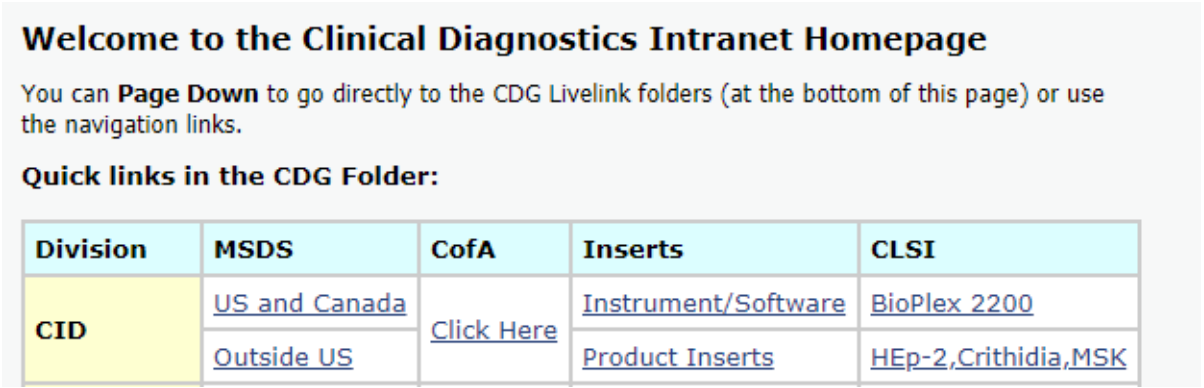
- **Control Data File**

http://livelinkprd.bio-rad.com/livelink/livelink.exe?func=ll&objId=77471385&objAction=browse&viewType=1

Supporting Tools

Clinical Diagnostics Intranet Homepage (CID)

- Product Insert (Reagent, CAL, Control)
- Certificate of Analysis
- MSDS



Welcome to the Clinical Diagnostics Intranet Homepage

You can **Page Down** to go directly to the CDG Livelink folders (at the bottom of this page) or use the navigation links.

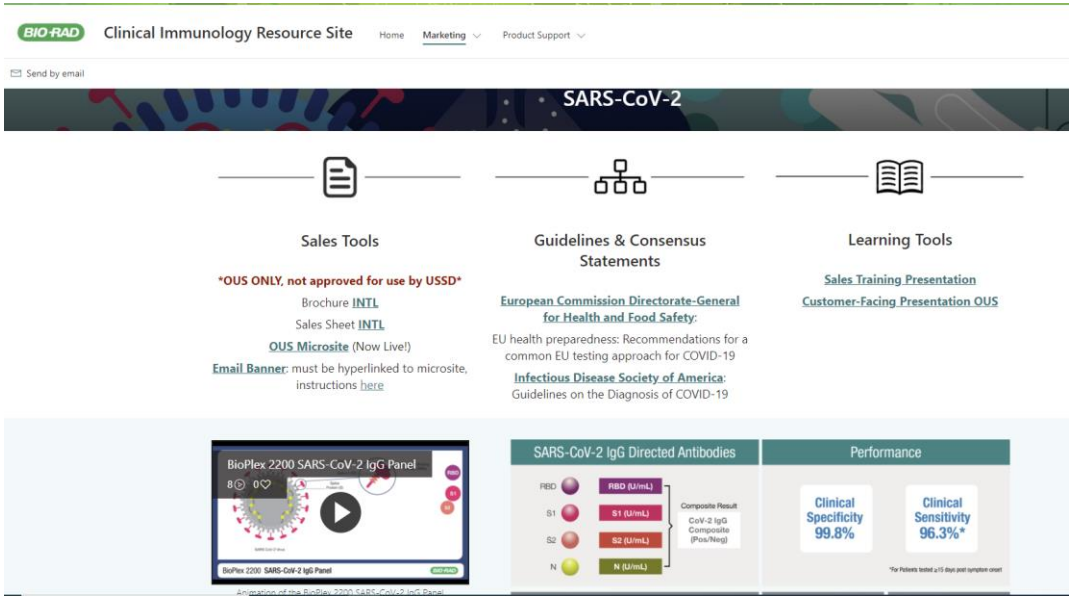
Quick links in the CDG Folder:

Division	MSDS	CofA	Inserts	CLSI
CID	US and Canada	Click Here	Instrument/Software	BioPlex 2200
	Outside US		Product Inserts	HEp-2,Crithidia,MSK

Documentation

- **Clinical Immunology Resource Site**

https://biorad.sharepoint.com/sites/ciresourcesite/SitePages/SARS-CoV-2.aspx



BIO-RAD

Proficiency Surveys

- **UK NEQAS**

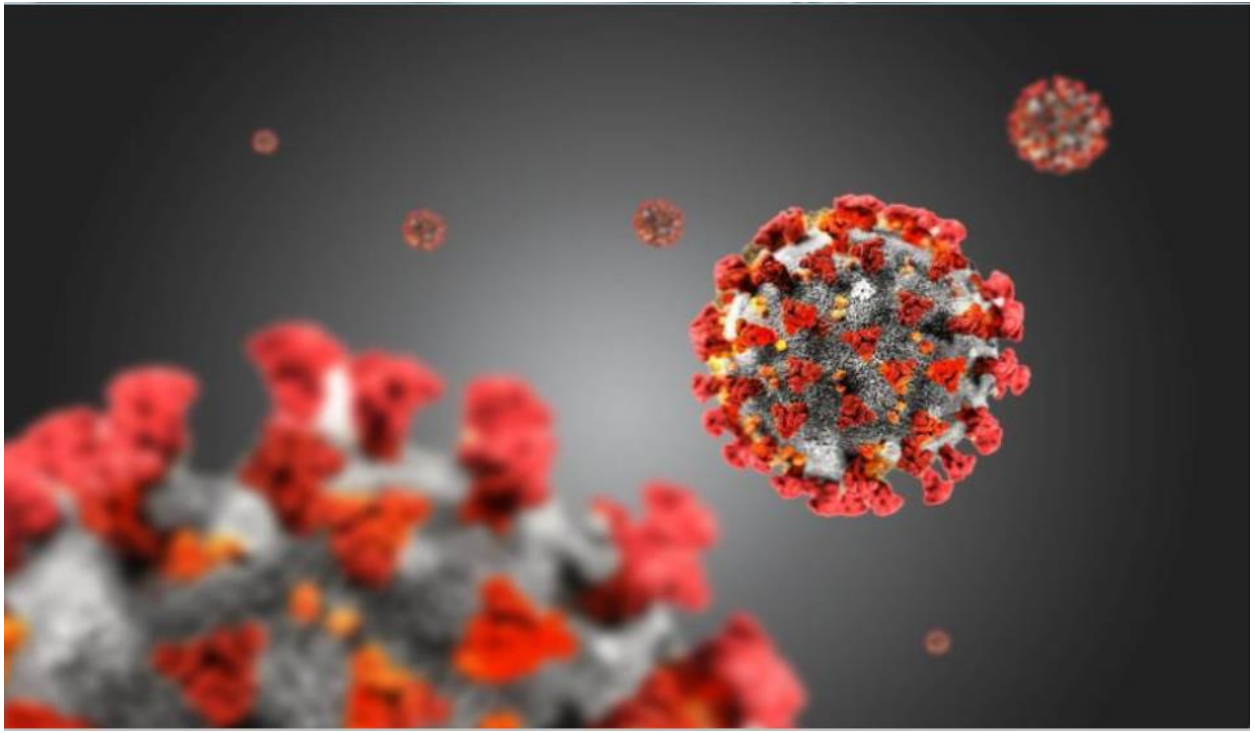
  SARS-CoV-2/Covid 19 Antibodies EQA Scheme

  Two distributions
- **College of American Pathologists** (USA)

  SARS CoV-2

  COVS (A,B)

Questions?



Post Training

Training Log Sheet: sign, date and return log sheet to Mary_Ann_Saunders@bio-rad.com