

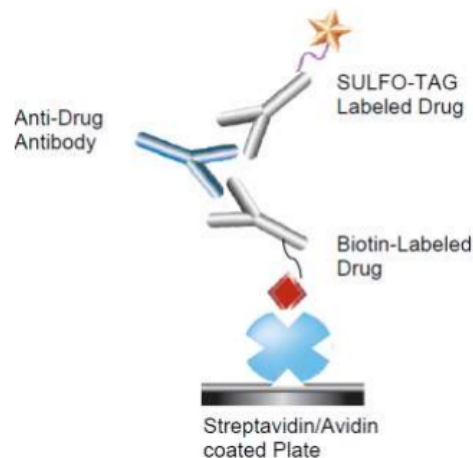
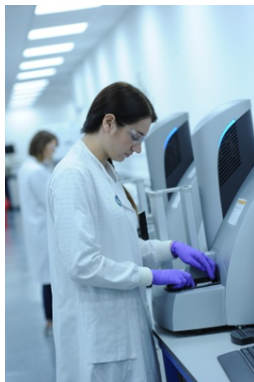
New immunogenicity strategies to meet the needs of a developing pandemic

Richard Hughes, Assoc. Sci. Director, Large Molecule Bioanalysis, LGC Fordham

(Unwanted) Immunogenicity – the anti-drug antibody format



MSD Bridging assay (ECLIA) - commonly used in the assessment of unwanted immunogenicity



Meso Scale Discovery®, Bridging Immunogenicity Assays

Why not an ELISA?

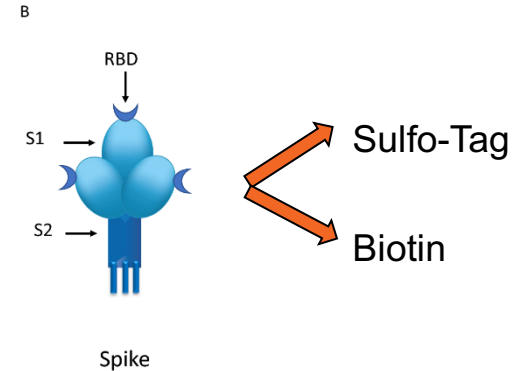
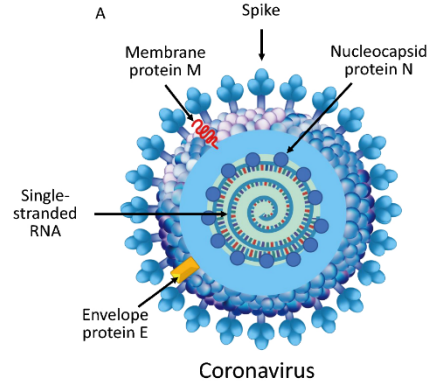
- Better sensitivity and analytical working range
- Homogenous solution phase incubation simplifies workflow
- Shorter assay times – higher throughput
- Not species specific



Early challenges in method development



- Early prototype assays used S1 and RBD fragments to investigate bridging assay potential
- Full length Spike protein was not available until June/July 2020
- Full length assays required additional development to reduce background
 - > Buffer optimization
 - > Different challenge ratios
 - > Concentration in assay
- Positive controls not specific (only cross reactive from SARS)



The Virus Itself
Rossi, et al. (2020) *Infection* volume 48, p.665–669

Final assay – format and precision

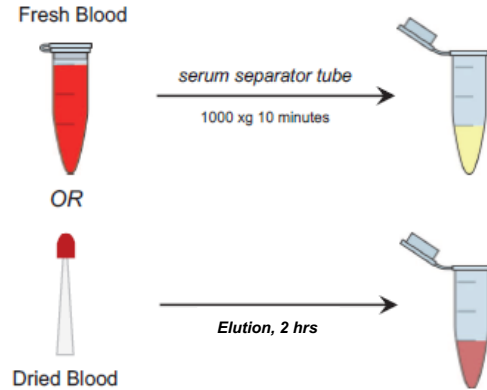
- Block Streptavidin MSD Plate
- Dilute sample 1 in 20
- Diluted sample is incubated for 1 hour with master mix containing equal concentrations of biotinylated- and sulfo-tagged full length spike protein
- Reaction mix is added to blocked plate for 1 hour
- Plate is washed and read on sector imager
- All liquid handling performed on an Integra ViaFlo



	left	middle	right
NC	146	145	135
CP-PC	1238	1299	1223
PC	5524	5462	4915
EPC	72557	73614	67643
NC	154	158	150
CP-PC	1326	1318	1213
PC	5408	5392	5088
EPC	73628	71590	70321

	Mean (n=6)	%CV
NC	148	5.4
CP-PC	1270	4.0
PC	5298	4.5
EPC	71559	3.2

Sample types – serum and WB micro-sample



Neoteryx Mitra® VAMS and collection kits

Benefits

At home sampling, no need for a clinic visit or venipuncture

CE Marked, FDA Class 1 devices

Devices can be shipped directly to us

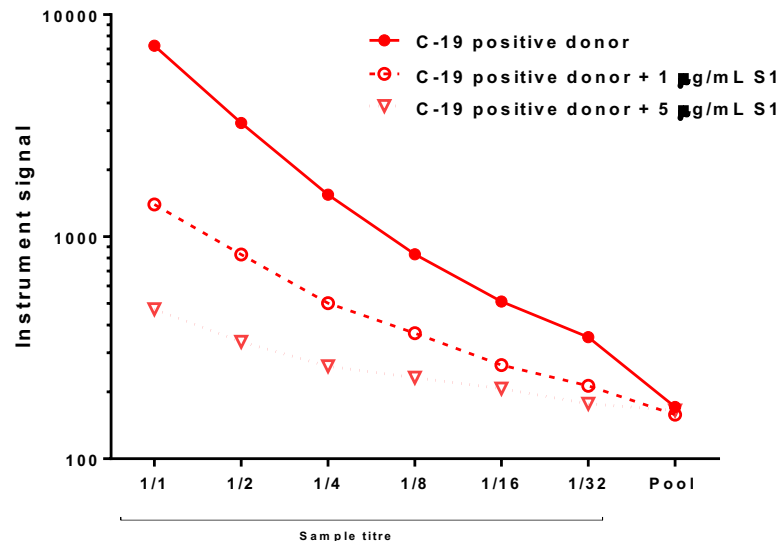
Barcoded, logged straight into our LIMS system for chain of custody and ease of reporting



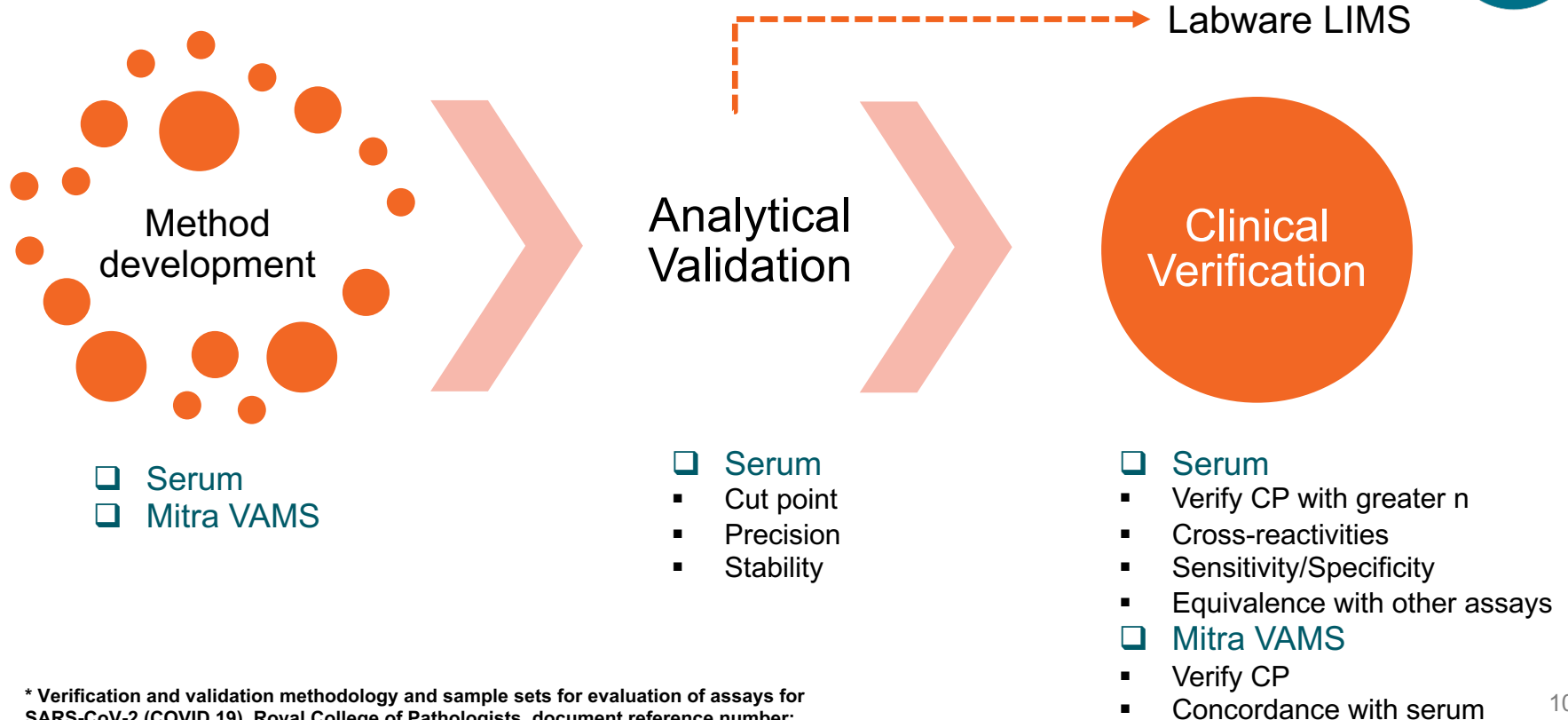
Questions over approach



- Do we need a tiered approach? What about confirmatory analysis??
- What about how we normally validate an ADA assay? Selectivity etc..
- Analytical Sensitivity, PCs are not as good as real positive samples – can we justify not having the 100 ng/mL box ticked?
- Should we not be analyzing in duplicate?
- What regulations should we be working to?

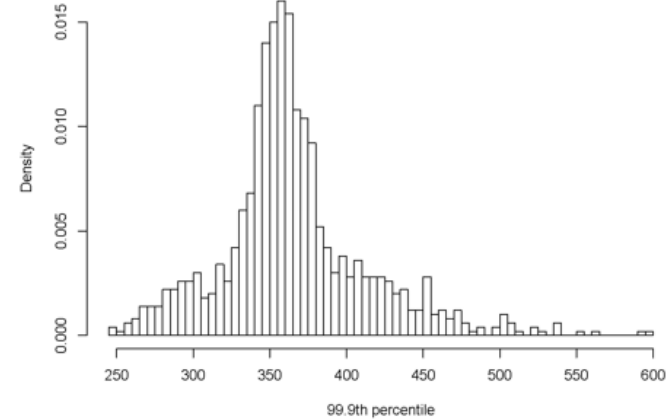
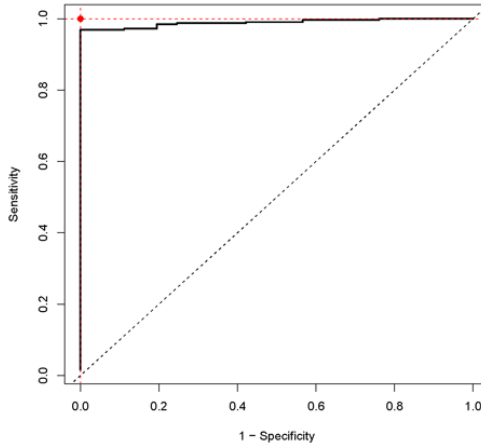
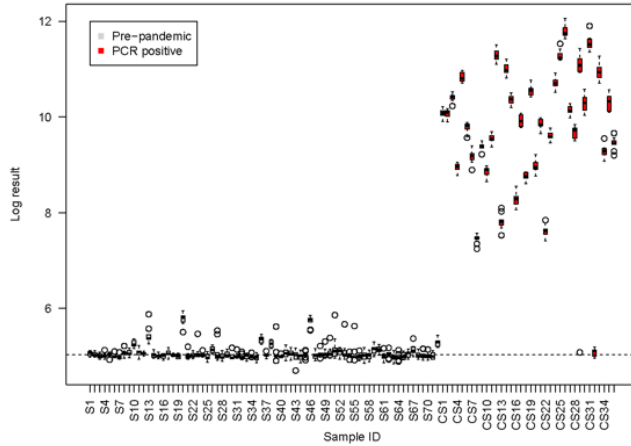


The pathway to assay roll out*



* Verification and validation methodology and sample sets for evaluation of assays for SARS-CoV-2 (COVID 19), Royal College of Pathologists. document reference number: G222-3 (2020).

Assay Validation - Cut point establishment



Given the purpose of the test, the threshold was set such that the specificity is as close to 100% as possible, while maximising the sensitivity. The point on the curve which is closest to the top left corner is at specificity 1 and sensitivity 0.9691, which is attained at thresholds between 385 and 1,400 RFU.

ROC Curve, the red dot represents a perfect test with 100% specificity and sensitivity

Bootstrapped values for the 99th percentile of the distribution of pre-pandemic samples

Assay Validation – Precision & Stability

- Precision – Intra/Inter-run and inter-analyst (3 analysts, 6 runs each of three plates)

Serum		NC	PC1	PC2	PC3
	Mean	115	973	4181	56106
	Std Dev.	16.6	169	741	10886
	CV%	14.5	17.4	17.7	19.4
	n	156	156	156	138

Mitra Eluate		NC	PC1	PC2	PC3
	Mean	112	1057	4587	19982
	SD	18.5	181	817	4566
	CV%	16.4	17.1	17.8	22.9
	n	80	80	80	80

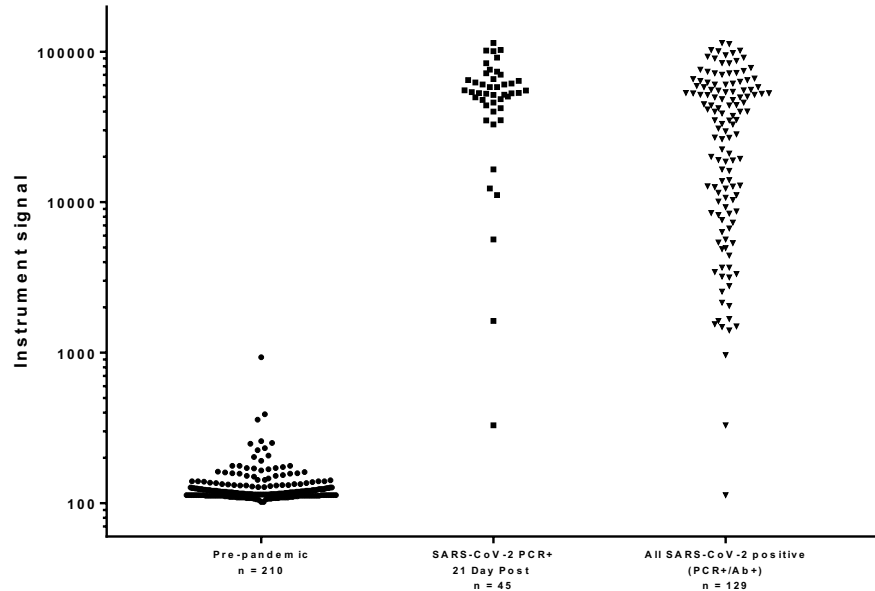
- PC1 and PC2 = Seracare Accurun controls. PC3 is high responding clinical sample
- All plate values are normalized to PC1, hence a positive sample is >1.

- Stability
 - Serum
 - Benchtop 24 hrs, 3 x Freeze/Thaw, Long term frozen at -80°
 - Mitra sample
 - Dried tip stability at RT and 35°C for 7 days (covers postage period)
 - Eluate - Benchtop 24 hrs, 3 x Freeze/Thaw, Long term frozen at -80°

Serum clinical verification



Bridging assay to detect SARS-CoV-2 antibodies
using Full-length Spike Protein



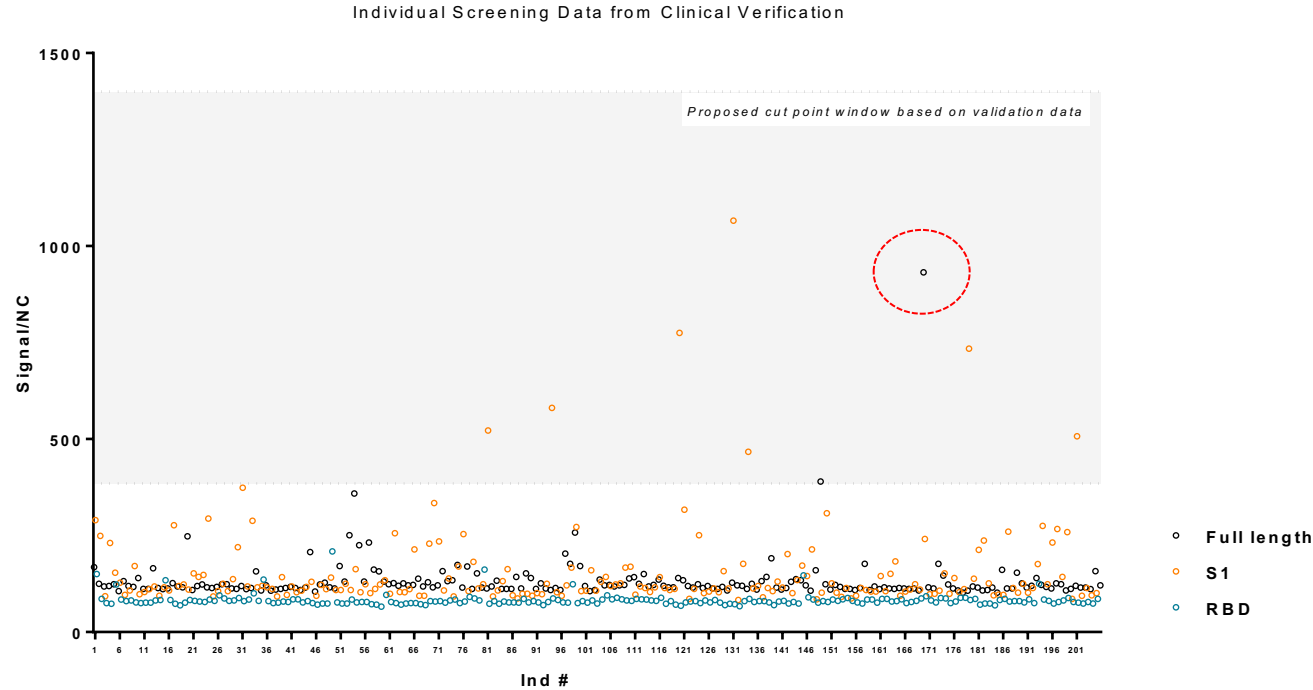
Days post PCR confirmation	N	Reactive	Non-Reactive	% Positive	Sensitivity % (95% CI)
11-20	19	19	0	100	
21-30	44	43	1*	98	
31-50	1	1	0	100	
From 21 days	45	44	1*	98.0	88.4 – 99.6

The assessment of sensitivity was performed on a cohort of COVID-19 patient samples where infection by SARS-CoV-2 had been confirmed by a PCR test 21 days prior to the sample being taken. In this case the assay demonstrated 98% Sensitivity.

Positive Ab samples by Comparator assay	N	Positive by LGC assay	Negative by LGC assay
Roche Elecsys Anti-Sars-CoV-2 serology assay	47	47	0
Abbot SARS-CoV-2 IgG assay	43	42	1*
Siemens SARS-CoV-2 Total (COV2T) assay	67	67	0

* Sample confirmed as Ab negative by both Roche Elecsys and Siemens assays

Have we got the cut point in the right place?



All samples were collected in 2018-2019

Serum clinical verification

- Assessment of serum samples for specificity used 377 pre-pandemic samples including the following disease state or interference assessments

Confounder samples

- 39 Coronavirus HKU Ab+
- 39 Coronavirus OC43 Ab+
- 40 Coronavirus 229E Ab+
- 38 Coronavirus NL63 Ab+
- 4 Parainfluenza Ab+
- 4 Influenza A Ab+
- 4 Influenza B Ab+
- 4 Respiratory Syncytial Virus Ab+
- 2 Rheumatoid Factor
- 2 HIV+
- 4 Enterovirus Ab+
- 31 EBV Nuclear Antigen positive
- 24 CMV Ab+
- 16 HBs Ab+
- 2 Immune thrombocytopenia (ITP)

Interference samples

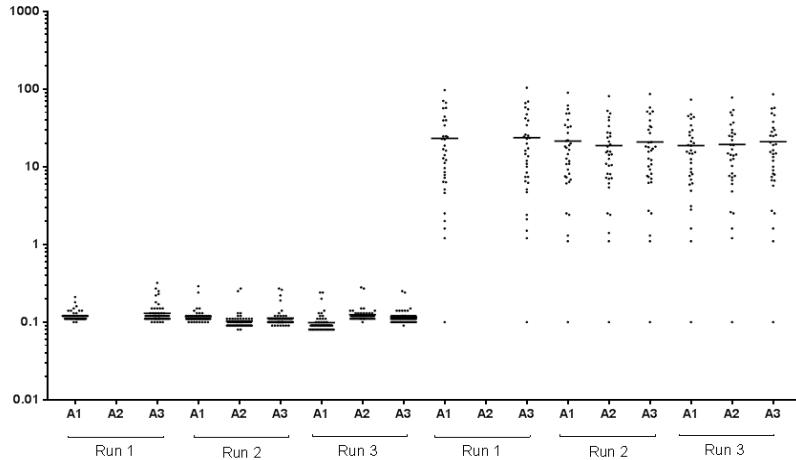
- 2x hyperlipidaemia patient samples
- 2x hyperlipidaemia (spiked to 4mg/mL)
- 2x hyperbilirubinaemia (spiked to 30 µg/mL)
- 2x haemolysed (3% equivalent to (>250 mg/dL of free haemoglobin)
- 2x Biotin (spiked to 1200 ng/mL)

Category	N	Reactive	Non-Reactive	Specificity (%)	95% CI
Negative samples: Pre-COVID era	301	0	301	100	
Interference samples	10	0	10	100	
Confounder samples	66	0	66	100	
Total	377	0	377	100	98.7 – 100%

Additional Mitra Eluate clinical verification

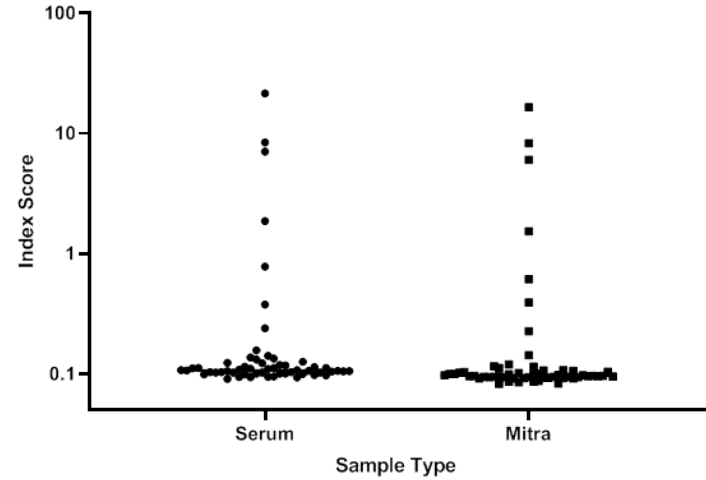


Comparison between serum values and surrogate mitra samples (comprising of red blood cells from a healthy donor combined with serum from pre-pandemic or confirmed COVID-19 patients).



Paired venous draw serum and capillary “finger prick” Mitra samples, from volunteers at LGC, were assessed for concordance.

Paired samples for SARS-CoV-2 Ab assay
(n=56)



Labware LIMS



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Covid-19 Home

FOR:LBL-01-TXT

9

2020

Receive Samples to Dilution Batches

Missing Sample Report

Manage Sample Sets

Manage Kits

Manage Assignments

Toggle Visibility

Columns

visibility

Print

Dilution Batches

Primary Batch ID	Client	Primary Station	Created By	Created On	Primary Samples	Primary Kit	View Primary	Backup ID	Backup Status	Backup Samples	Backup Kit	View Backup	Confirm	Cancel
COVID_020-08-057	Any	ASSIGNED	ABHI.BANDEKAR	25/AUG/20 14:16:30	20	COVID_020-08-057	View	COVID_020-08-057	STORE ONLY	19		View		
COVID_020-08-058	Any	ASSIGNED	ABHI.BANDEKAR	25/AUG/20 14:14:14	21	COVID_020-08-058	View	COVID_020-08-058	STORE ONLY	20		View		
COVID_020-08-059	Any	ACCEPTED	ABHI.BANDEKAR	25/AUG/20 15:37:54	3	COVID_020-08-059	View	COVID_020-08-059	AWAIT DISPOSE	3		View		
COVID_020-08-060	Any	PLANNED	ABHI.BANDEKAR	27/AUG/20 09:50:14	0	COVID_020-08-060	View	COVID_020-08-060	PLANNED	0		View		
COVID_020-08-061	Any	PLANNED	ABHI.BANDEKAR	27/AUG/20 09:50:14	0	COVID_020-08-061	View	COVID_020-08-061	PLANNED	0		View		

Build Instrument Batch

Columns

visibility

Print

Instrument Batches

Batch ID	Client ID	Client	Created On	Status	Run Date	Analyst	File Path	Export	Import	Clear	View	Cancel
COVID_020-08-005	COVID-20-08-059	Any	26-AUG-20	ABHI.BANDEKAR/ACCEPTED	26-AUG-20	ABHI.SCI	//Client/CE/Users/abhi.bandekar/Documents/Abhi/COVID_020-08-005_ABHI_26_AUG_2020.txt				View	Run
COVID_020-08-006	COVID_020-08-059	Any	26-AUG-20	ABHI.BANDEKAR/RESULTS ENTERED	26-AUG-20	WALDA.KAYE	//Client/CE/Users/abhi.bandekar/Documents/Abhi/COVID_020-08-006_ABHI_26_AUG_2020.txt				View	Run
COVID_020-08-008	K2	Any	27-AUG-20	OWEN.CROSS/RESULTS ENTERED	27-AUG-20	ALICE.COUSINS	//Client/CE/LW QuickAccess/Covid Prototype Files/COVID_020-08-008_OWEN_CROSS_26_AUG_2020.txt				View	Run
COVID_020-08-009	TESTING 96 WELL GEN	Any	27-AUG-20	OWEN.CROSS/CONFIRMED	27-AUG-20	ALICE.COUSINS	LGC305183Q820 RUN006_1_10_HAR-18 114007_100.txt				View	Run
COVID_020-08-010	96 WELL GEN TEST 2	Any	27-AUG-20	OWEN.CROSS/RESULTS ENTERED	27-AUG-20	ALICE.COUSINS					View	Run
COVID_020-08-011	16S1	Any	27-AUG-20	OWEN.CROSS/CONFIRMED	27-AUG-20	ALICE.COUSINS					View	Run
COVID_020-08-012	COVID_020-08-062	Any	28-AUG-20	OWEN.CROSS/REJECTED	28-AUG-20	ALICE.COUSINS	//Client/CE/LW QuickAccess/Covid Prototype Files/COVID_020-08-012_OWEN_CROSS_28_AUG_2020.txt				View	Run

This is the plate review screen after data import. On the right hand side we have the plate level data.

Top table shows the cut point control data.

Middle table is the Positive and Negative QC data.

At the bottom we find the unknown sample results.

This is the Covid Home screen, the workflow is as follows:

- Create new clients/sites as required
- Upload Mitra tip kit barcodes
- Linking a shipment with a client.
- Sample batching and QC checking
- After analysis - run reviewed, accepted or rejected.
- Reporting

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Covid-19 Run Review

FOR:LBL-01-TXT

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2020

Run: COVID_020-08-006 | Date of Analysis: 26-AUG-20 | Analyst: WALDA.KAYE

Accept Run

Reject Run

Plate Data

CPC Mean: 6.6	CPC CV%: 0.6
NC Mean: 104.8	NC CV%: 104.8
NC SD: 11.3	NC Range: 50-220
PC Mean: 995.5	PC CV%: 995.5
PC SD: 6.3	PC Range: 750-1250
Warnings	None

Cut Point Controls

ID	Type	Pos	Med	Response	Cmd	Comment	Add Comment	Reject	Reason
COVID_QC_CPC-38861	Covid 19 Negative Control	2 No	100 Yes	0.1 Negative	Add			Reject	
COVID_QC_CPC-38864	Covid 19 Negative Control	4 No	99 Yes	0.1 Negative	Add			Reject	
COVID_QC_CPC-38867	Covid 19 Negative Control	47 No	102 Yes	0.1 Negative	Add			Reject	
COVID_QC_CPC-38870	Covid 19 Negative Control	48 No	102 Yes	0.1 Negative	Add			Reject	
COVID_QC_CPC-38873	Covid 19 Negative Control	63 No	102 Yes	0.1 Negative	Add			Reject	
COVID_QC_CPC-38876	Covid 19 Negative Control	56 No	102 Yes	0.1 Negative	Add			Reject	

QC Samples

ID	Type	Pos	Med	Response	Cmd	Comment	Add Comment	Reject	Reason
COVID_QC_NC-38860	Covid 19 Negative Control	2 No	100 Yes	0.1 Negative	Add			Reject	
COVID_QC_NC-38863	Covid 19 Negative Control	5 No	100 Yes	0.1 Negative	Add			Reject	
COVID_QC_NC-38866	Covid 19 Negative Control	44 No	99 Yes	0.1 Negative	Add			Reject	
COVID_QC_NC-38869	Covid 19 Negative Control	47 No	102 Yes	0.1 Negative	Add			Reject	
COVID_QC_NC-38872	Covid 19 Negative Control	52 No	93 Yes	0.2 Negative	Add			Reject	
COVID_QC_NC-38875	Covid 19 Negative Control	3 No	100 Yes	0.1 Negative	Add			Reject	
COVID_QC_NC-38878	Covid 19 Positive Control	4 No	1010 Yes	1.3 Positive	Add			Reject	
COVID_QC_NC-38881	Covid 19 Positive Control	43 No	995 Yes	1.3 Positive	Add			Reject	
COVID_QC_NC-38884	Covid 19 Positive Control	46 No	1002 Yes	1.3 Positive	Add			Reject	
COVID_QC_NC-38871	Covid 19 Positive Control	91 No	993 Yes	1.3 Positive	Add			Reject	
COVID_QC_NC-38874	Covid 19 Positive Control	94 No	993 Yes	1.3 Positive	Add			Reject	

Validation Samples

ID	Type	Pos	Med	Response	Cmd	Comment	Add Comment
2050A	Covid Primary Sample	7 No	100	0.1 Negative	Add		
2051A	Covid Primary Sample	8 No	100	0.1 Negative	Add		
2052A	Covid Primary Sample	9 No	100	0.1 Negative	Add		
2053A	Covid Primary Sample	10 No	75	0.1 Negative	Add		
2054A	Covid Primary Sample	11 No	123	0.2 Negative	Add		
2055A	Covid Primary Sample	12 No	1430	1.3 Positive	Add		
2056A	Covid Primary Sample	13 No	100	0.1 Negative	Add		
2057A	Covid Primary Sample	14 No	100	0.1 Negative	Add		
2058A	Covid Primary Sample	15 No	100	0.1 Negative	Add		
2059A	Covid Primary Sample	16 No	900	1.2 Positive	Add		
2060A	Covid Primary Sample	17 No	230	0.3 Negative	Add		
2061A	Covid Primary Sample	18 No	240	0.3 Negative	Add		

All data is fake data created for testing so it may be inconsistent

7

Whats next...



The MHRA are very clear that an assay such as this would be classed as a diagnostic medical device.

As such, it requires a CE mark (**done**) and performance under an ISO15189 or ISO17025 quality system (**pending inspection**).

» UKAS position on accreditation of COVID-19 testing under ISO/IEC 17025:2017

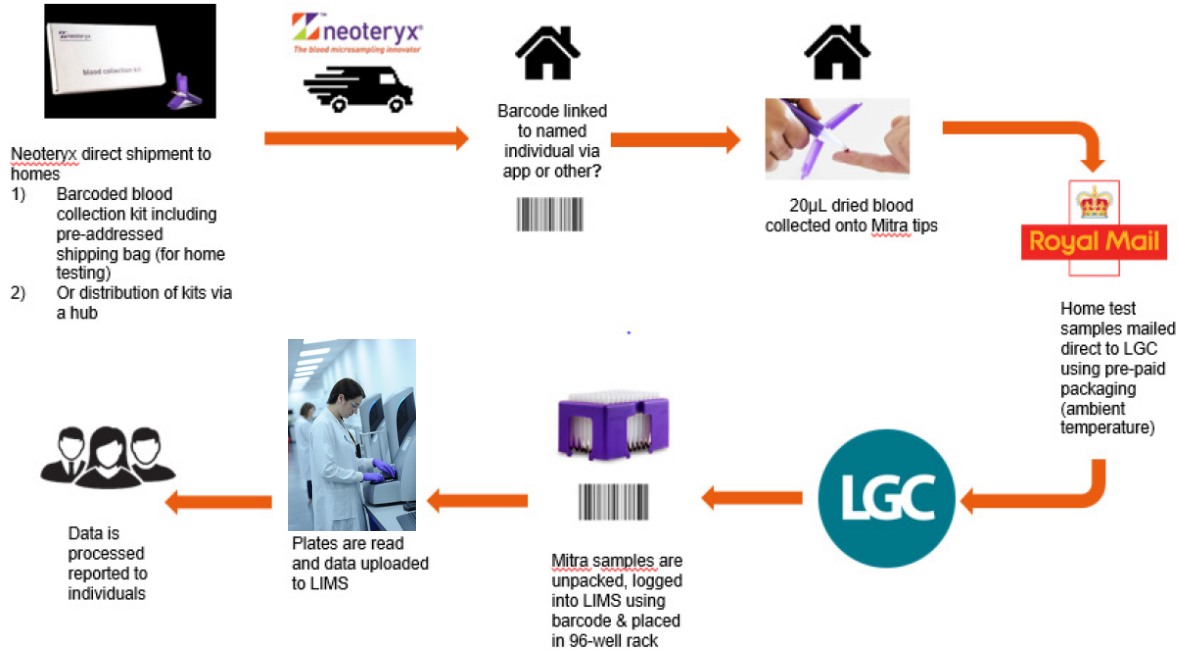
27 July, 2020



Background

Laboratories accredited to ISO 15189:2012 or ISO/IEC 17025:2017 can apply to include COVID-19 testing in their accreditation, using the AC COV form. It is acknowledged that to increase the national testing capacity, it may be necessary for non-clinical laboratories accredited to ISO/IEC 17025:2017 to be utilised. UKAS believes that there are certain circumstances where this may be entirely appropriate to do and this position is confirmed by the UKAS MedLabs Technical Advisory Committee (TAC). The TAC provides technical advice to UKAS on matters relating to the development and operation of the accreditation process. This position statement is to clarify the circumstances under which ISO/IEC 17025:2017 accreditation would be appropriate.

An end-to-end solution (its not all about the assay...)



Thank you for listening



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With thanks to:

Laura Geary
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