New immunogenicity strategies to meet the needs of a developing pandemic

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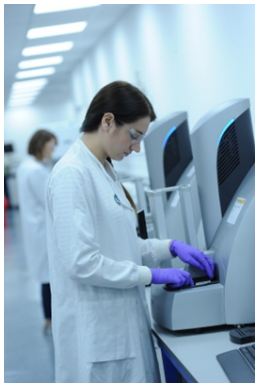
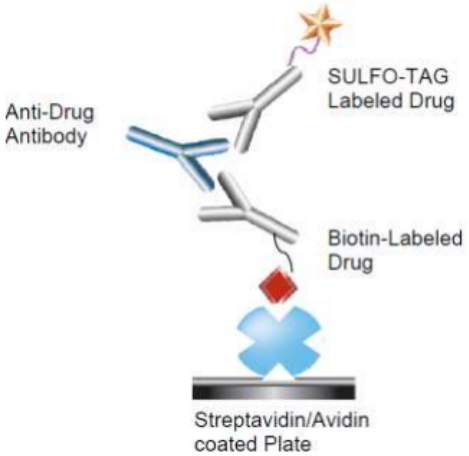
(Unwanted) Immunogenicity – the anti-drug antibody format

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





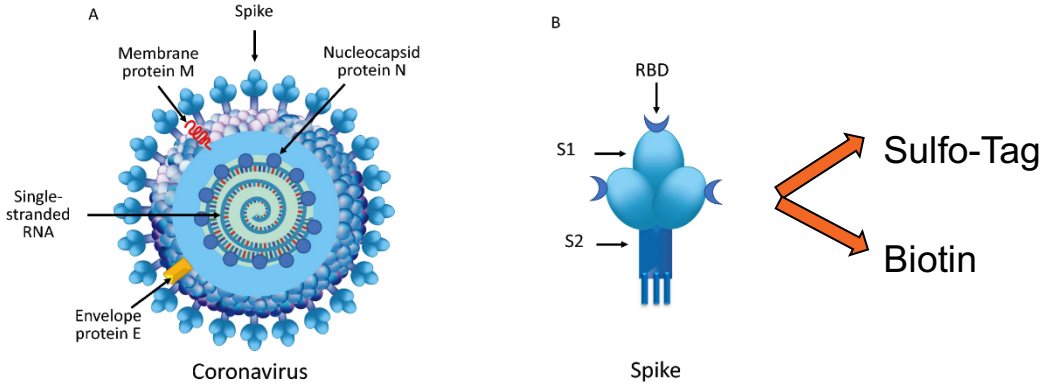
Meso Scale Discovery®, Bridging Immunogenicity Assays

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

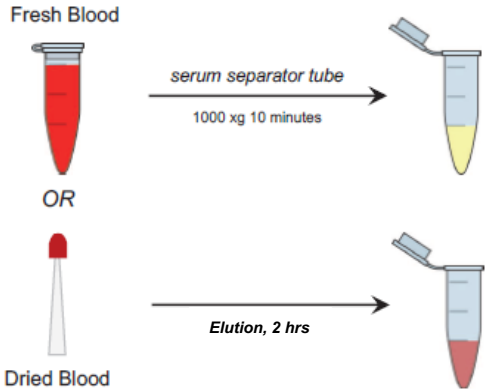
Final assay – format and precision

- 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 an 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

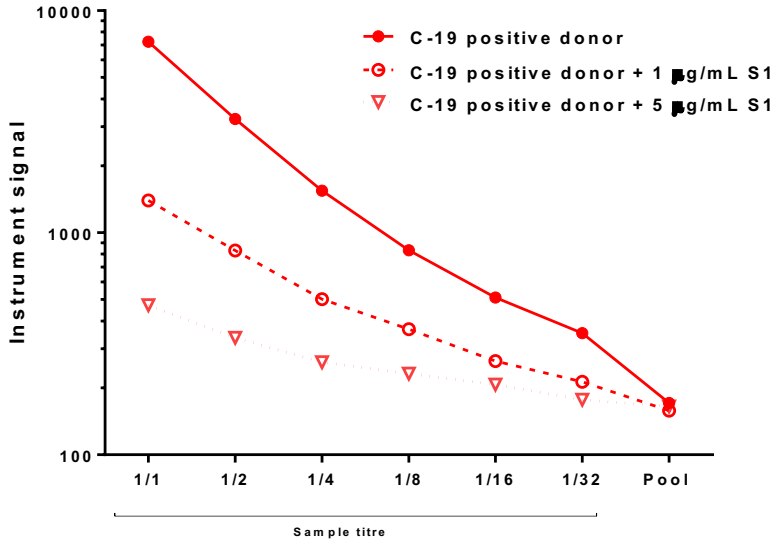
CE Marked, FDA Class 1 devices

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*

Labware LIMS

- ❑ Serum
- ❑ Mitra VAMS

Analytical Validation

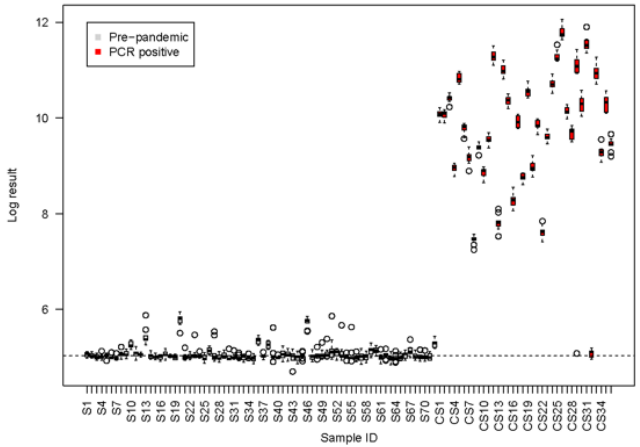
- ❑ Serum
  - Cut point
  - Precision
  - Stability

Clinical Verification

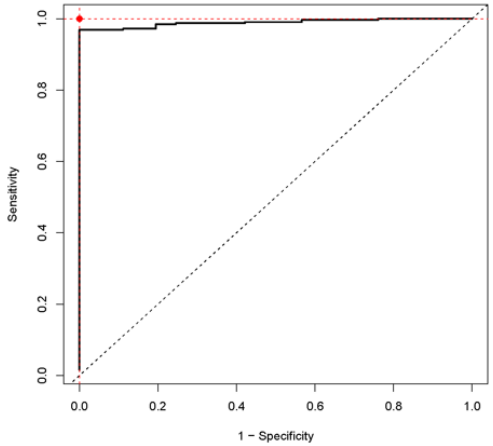
- ❑ Serum
  - Verify CP with greater n
  - Cross-reactivities
  - Sensitivity/Specificity
  - Equivalence with other assays
- ❑ Mitra VAMS
  - Verify CP
  - Concordance with serum

*** 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).**

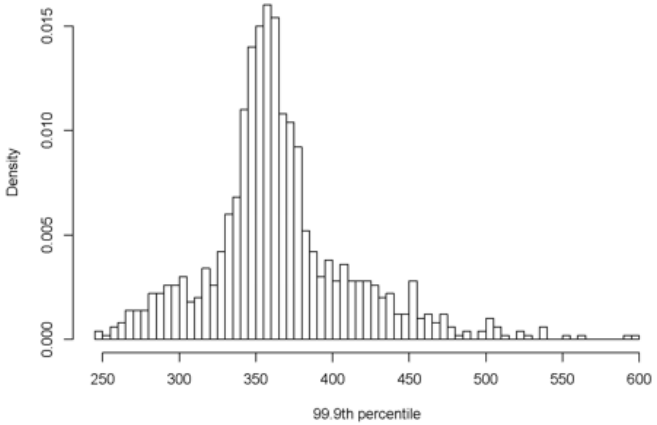
Assay Validation - Cut point establishment



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

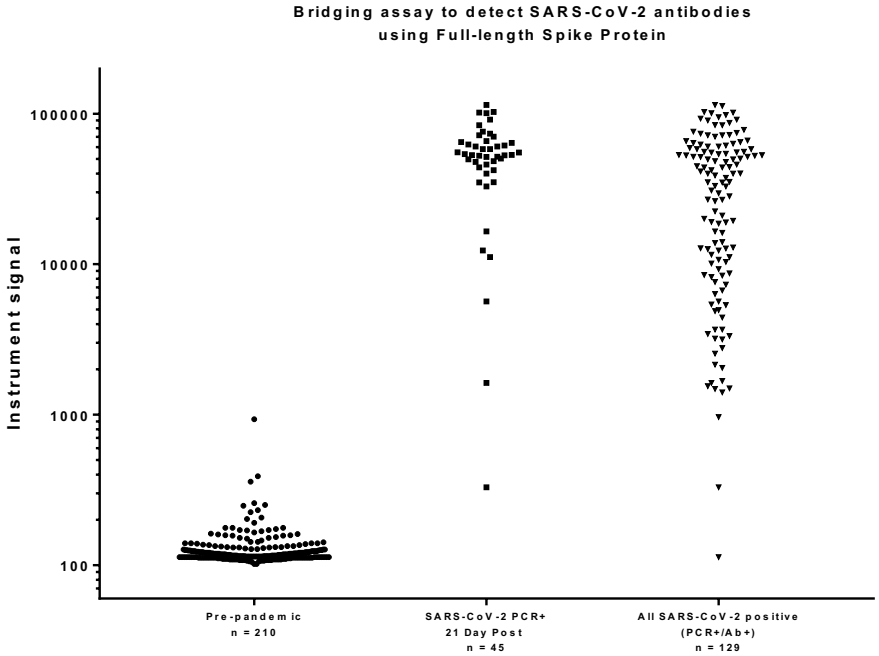
- Precision – Intra/Inter-run and inter-analyst (3 analysts, 6 runs each of three plates)

		NC	PC1	PC2	PC3
Serum	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
		NC	PC1	PC2	PC3
Mitra Eluate	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



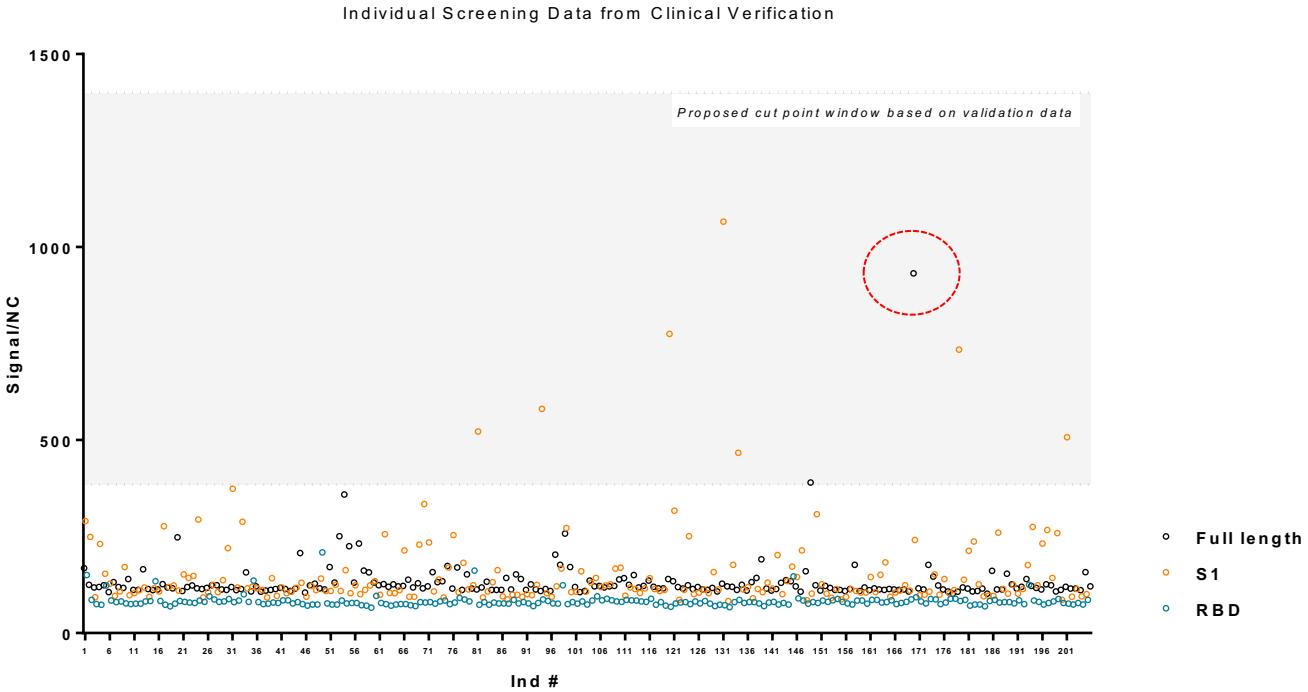
Days post PCR confirmation	N	Reactive	Non-Reactive	% Positive	Sensitivity % (95% CI)
11-20	19	19	0	100	
21-30	44	43	1*	98	
31-50	1	1	0	100	
From 21 days	45	44	1*	98.0	88.4 – 99.6

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

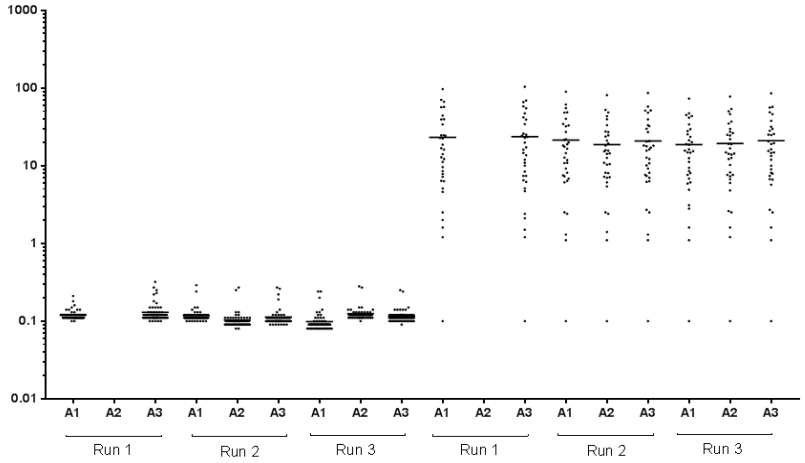
Confounder samples
- 39 Coronavirus HKU Ab+
- 39 Coronavirus OC43 Ab+
- 40 Coronavirus 229E Ab+
- 38 Coronavirus NL63 Ab+
- 4 Parainfluenza Ab+
- 4 Influenza A Ab+
- 4 Influenza B Ab+
- 4 Respiratory Syncytial Virus Ab+
- 2 Rheumatoid Factor
- 2 HIV+
- 4 Enterovirus Ab+
- 31 EBV Nuclear Antigen positive
- 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)

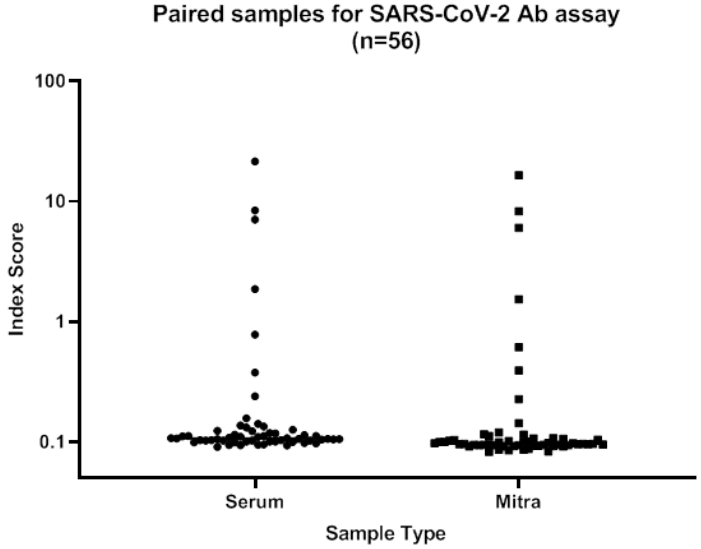
Category	N	Reactive	Non-Reactive	Specificity (%)	95% CI
Negative samples: Pre-COVID era	301	0	301	100	
Interference samples	10	0	10	100	
Confounder samples	66	0	66	100	
Total	377	0	377	100	98.7 – 100%

Additional Mitra Eluate clinical verification

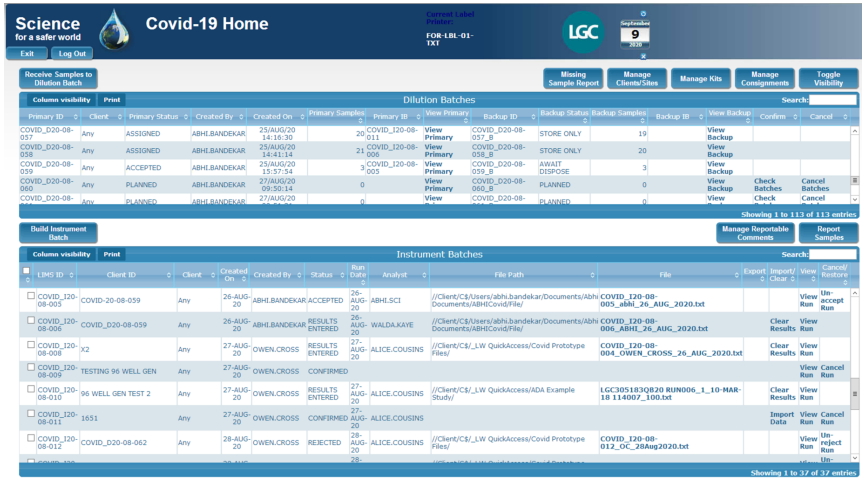
Comparison between serum values and surrogate mitra samples (comprising of red blood cells from a healthy donor combined with serum from pre-pandemic or confirmed COVID-19 patients).



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

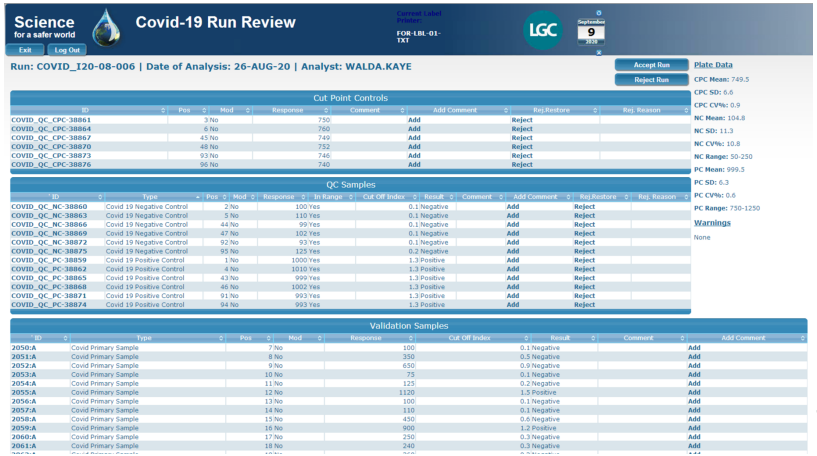


Labware LIMS



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



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.

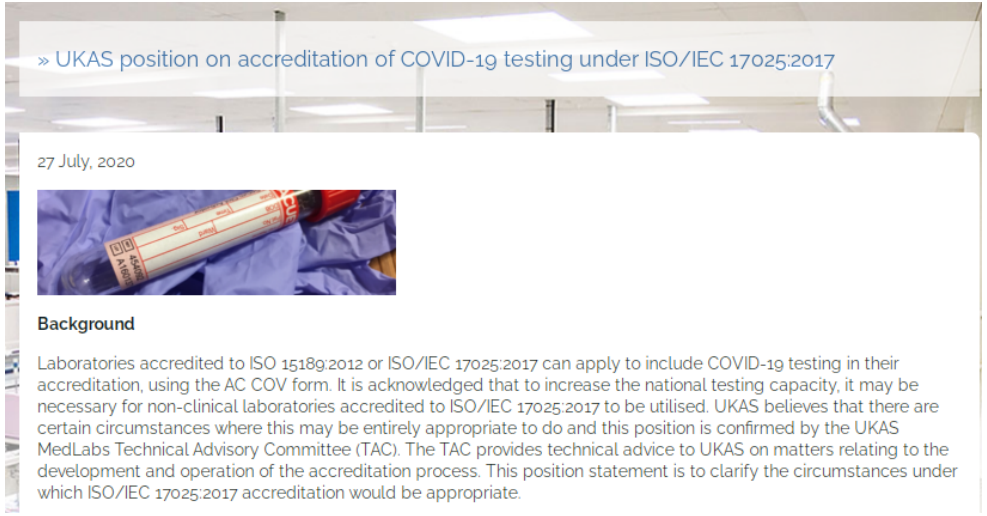
At the bottom we find the unknown sample results.

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

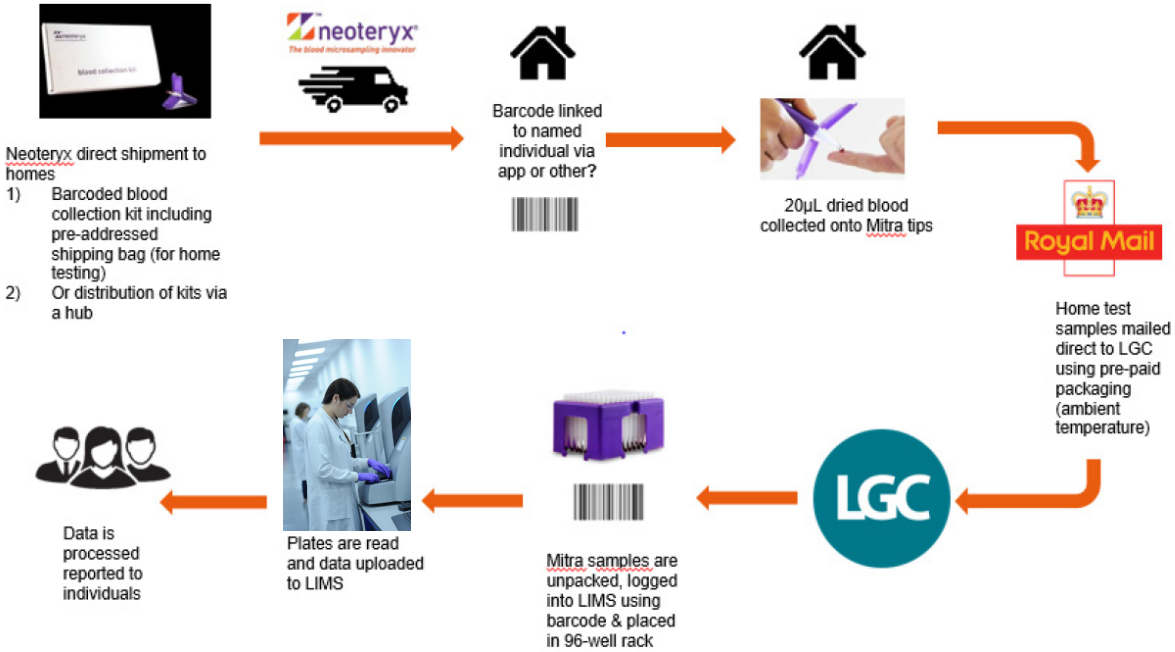
Whats next…

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).



An end–to–end solution (its not all about the assay…)



Thank you for listening

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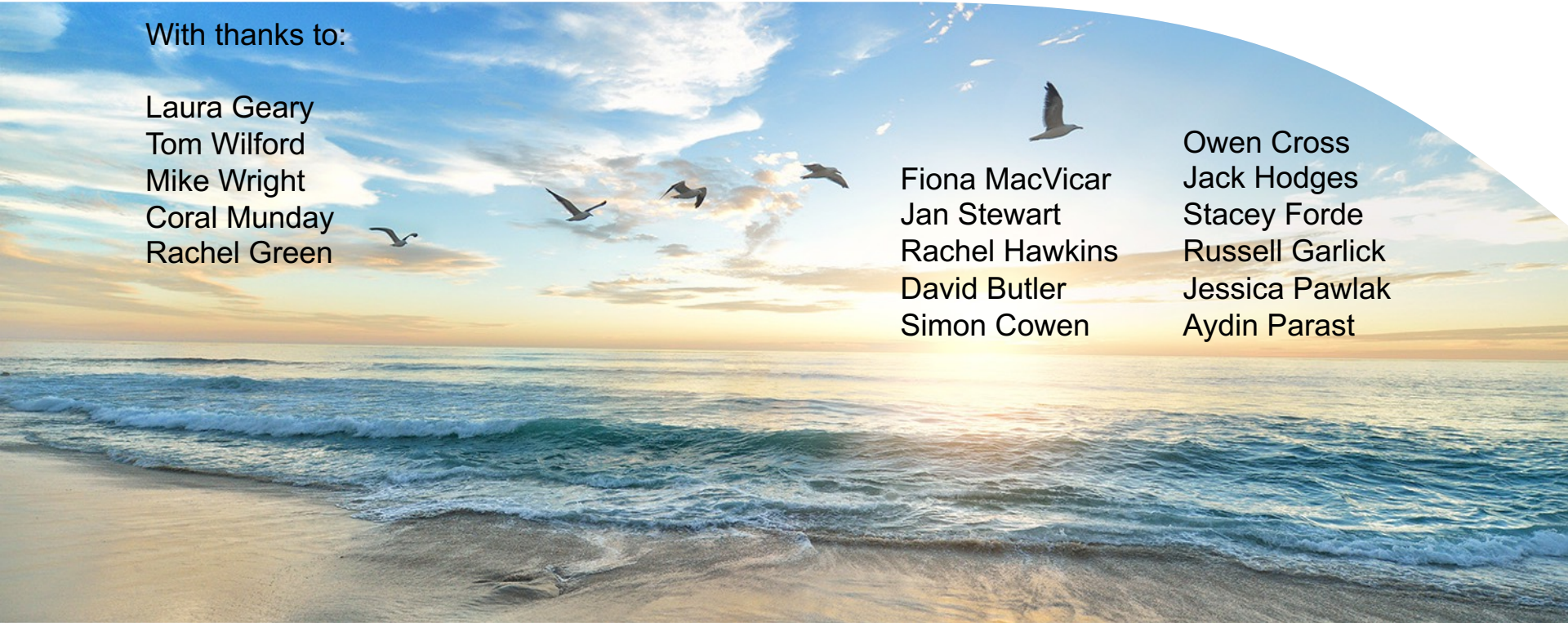

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