KRONO MID-TERM REVIEW 01/10/2021

KRONO

Evaluation of a production ready portable, Point of Need Platform (instrument and reagents), direct from nasal swab test for the molecular diagnostic detection of COVID-19 infection

« This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101005075. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA »

KRONO MEMBERS

Nelson Nazareth
BGR / BioGene, UK

Nathan Nazareth
David Edge

Rémi Charrel
AMU, France

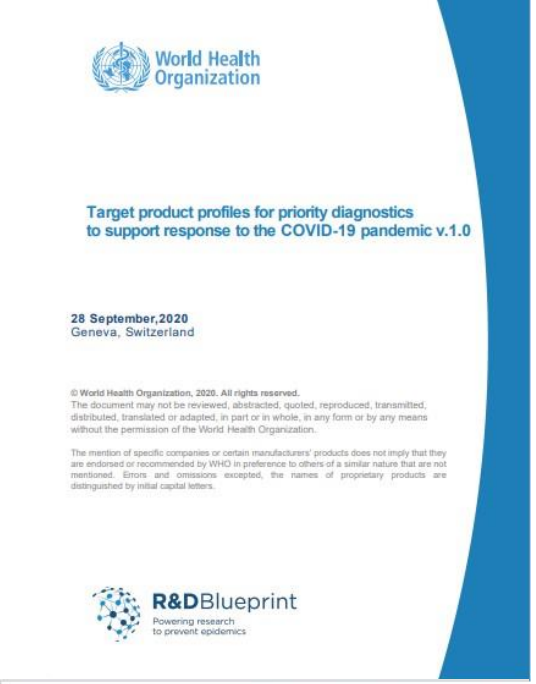
Margo Barthelemy
Thérèse Atieh
Nazli Ayhan Yilmaz
Sandy Gore

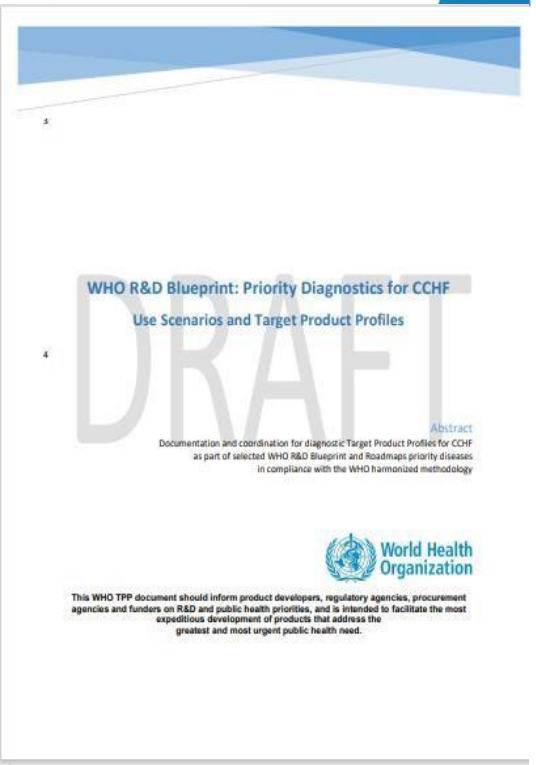
Giulia Matusalia
INMI, Italy

Claudia Minosse
Francesca Colavita
Rafaella Marconi
Benilde Tomei
Di Caro Antonino
Lalle Eleonora Concetta
Castilletti

Alessandra Falchi
UCPP, France

Lisandru Capai





KRONO KEY OBJECTIVES

- Meet the WHO R&D blueprint TPP of 10,000 virions/ml detection for simple to use, low cost tests for use in the developing world.
- Ultimately develop assays for all blueprint targets.
- Ability to deploy tests for use without lab access in remote regions and used by anyone with simple training.
- Drive the cost of goods to the point that assays can be sold at the price point of lateral flow but with sensitivity of molecular

KRONO KEY OBJECTIVES

- Advancement of the portable detection system from the existing lab-based technology demonstrator to a portable validated production u ready to be manufactured at scale to impact on both the curre pandemic and future outbreaks of emergent disease.

- Development and validation of the SARS-CoV-2 assay, including internal positive control – latterly becoming a Duoplex test.

- Demonstration of rapid development and scaling to production of the lyophilised assays, including enzyme production, reagent and lyophilisation development.

KRONO KEY OBJECTIVES

- Validation of the SARS-CoV-2 assay, initially using pseudovirus supplied by UK NIBSC and Armoured RNA produced by AMU, and latterly using cultured virus at INMI, Rome

- Performing a study of paired, blinded nasopharyngeal swab samples against a gold standard lab-based assay to show equivalency.

- Proof of concept for a direct from swab differential diagnostic between pan flu and SARS-CoV-2, the released and validated assay would be SARS-CoV-2 only due to having less regulatory burden for release(EUL route)

KRONO KEY OBJECTIVES

- Publication and dissemination of the project outcomes and preparation for scaling and WHO EUL approval.

Stretch Goal

- Designing a direct from whole blood assay to demonstrate the team's ability to rapidly respond to a future outbreak of disease X – Yellow Fever Virus was chosen as the exemplar target.

PROJECT OVERVIEW

- BG Research had previously demonstrated direct detection from blood and animal nasal swabs without any requirement for nucleic acid extraction using a closed tube, single enzyme reagent method.

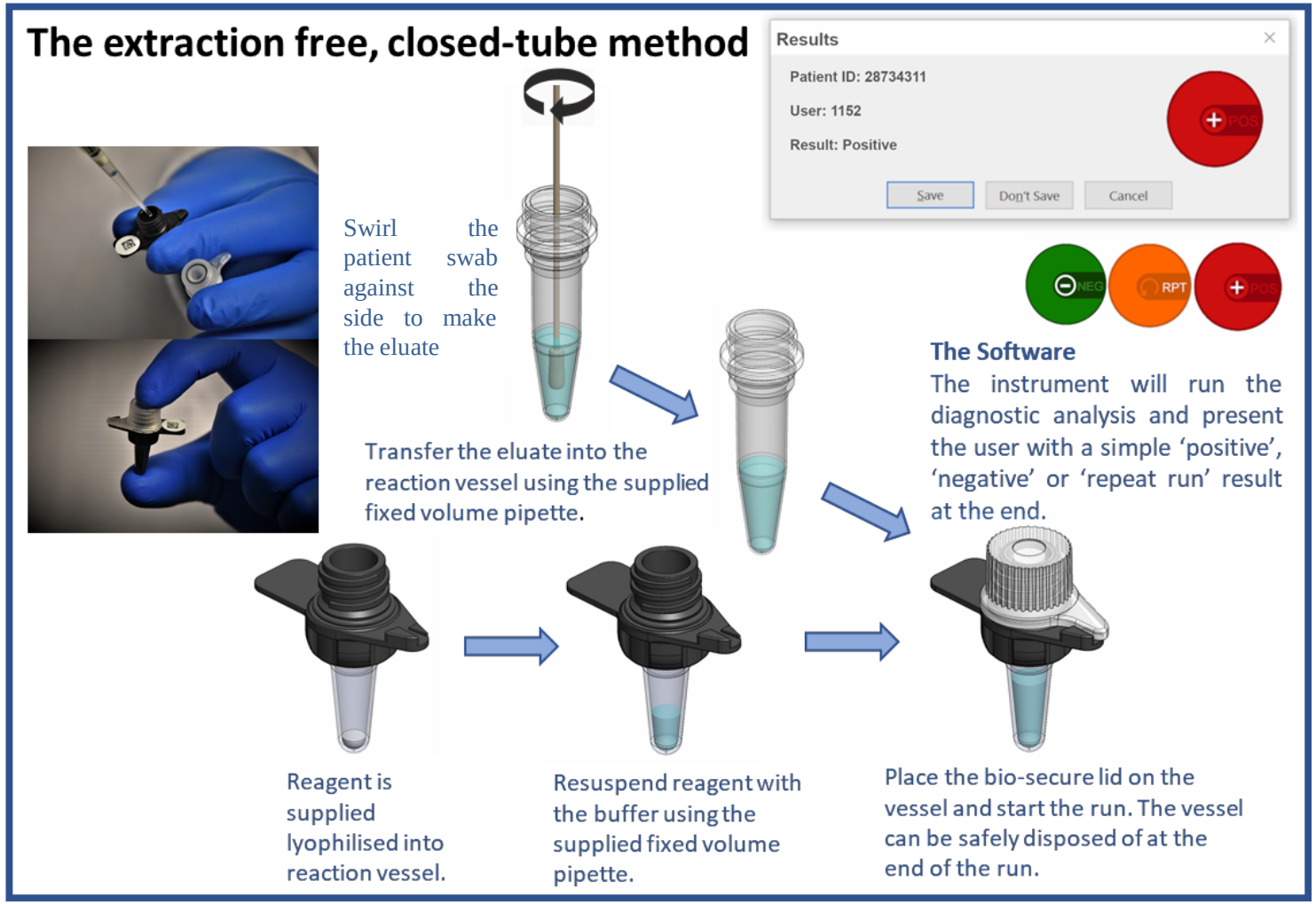
- Approach had demonstrated comparable results to lab- based reference assays from an extraction.
- BGR had a frozen reagent designed to amplify directly from whole blood but had not been tested with viral transport medium or human nasal swabs.
- BGR had built a technology demonstrator and reaction vessel for running the direct from crude sample process.

	BGR XF assay Ct Values						Reference assay Ct values					
Animal No.	197	7713	4276	4231	4235		Animal No.	197	7713	4276	4231	4235
0 dpc	No Ct	No Ct	No Ct	No Ct	No Ct		0 dpc	No Ct	No Ct	No Ct	No Ct	No Ct
2 dpc	32	29	No Ct	30	32		2 dpc	No Ct	31.5	No Ct	No Ct	No Ct
4 dpc	29	32	32	30	30		4 dpc	26	33.8	34	27.9	27.4
5 dpc	26	30	33	28	25		5 dpc	26.9	29.3	32.7	26	23
6 dpc	25	27	31	26	25		6 dpc	24.7	29.5	29.6	24	21.7
7 dpc	23	26	30	no sample	24		7 dpc	20.2	25.7	30	no sample	23.3
8 dpc	20	23	27	no sample	24		8 dpc	19.9	21.3	24.8	no sample	20.8
9 dpc	no sample	25	27	no sample	25		9 dpc	no sample	21	21.3	no sample	22.5
10 dpc	24	24	25	no sample	27		10 dpc	24	22.1	24	no sample	24.7
12 dpc	28	27	28	no sample	29		12 dpc	25.2	25.4	26	no sample	25.3
14 dpc	no sample	27	No Ct	no sample	30		14 dpc	no sample	25	24.9	no sample	25.4

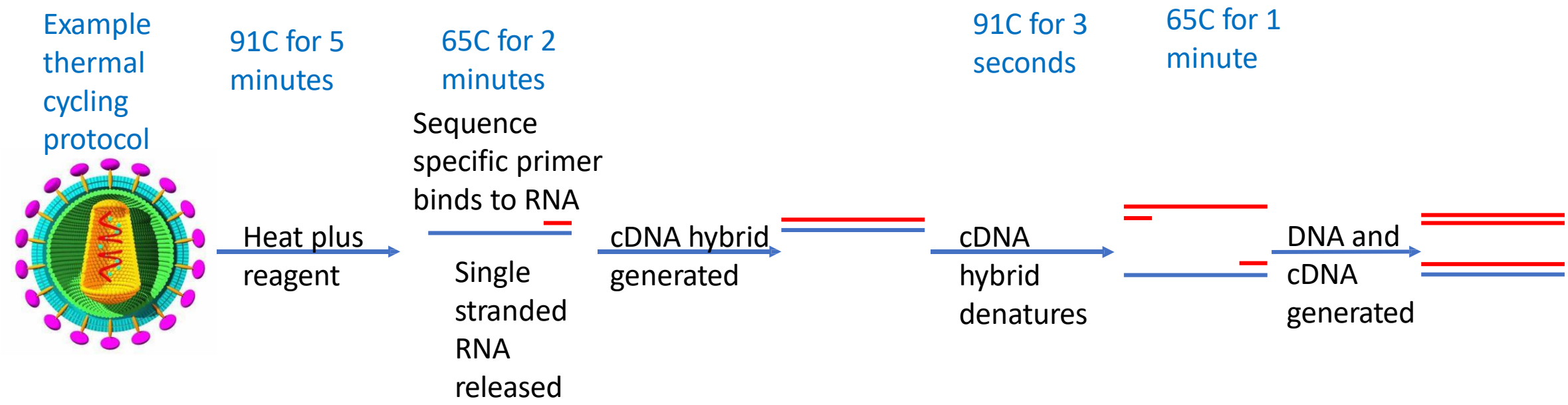
BGR PPRV assay direct from goat nasal swab resuspended into 1ml water. BGR method directly added 12ul of nasal swab eluate, the Batten PPRV (WHO FAO) reference assay was run from an extraction according to the protocol.

TECHNOLOGY SUMMARY

- Extraction free, direct from crude sample molecular testing
- Reagent lyses viruses
- Stabilises RNA
- Sequesters inhibitors
- Performs direct RT-QPCR from a wide range of crude samples including blood, saliva, nasal swab eluates and urine
- Instrument is low cost and portable
- Cold chain free and not requiring lab facilities or expert users



TECHNOLOGY SUMMARY



- Single enzyme performs DNA and RNA directed DNA polymerisation.
- Thermostable - >20 minutes half-life at 94C.
- Multiple rounds of RT are possible.
- Uses hydrolysis probes for sequence specific detection.

- Enzyme is non-native RT which cannot degrade template RNA
- Reagent stabilises the released viral genomic RNA
- Multiple opportunities to “catch” target when at low titre, gives reproducible Ct even near LLOD.

Progress, Achievements & Results

WP1 – progress achievements and results

- Held monthly minuted web meeting between the partners, all tasks actioned.
- Meeting reports filed with IMI2.
- Management procedures well established and working well – work package will be delivered as expected.

WP2 – progress achievements and results

- Designed a singleplex SARS CoV-2 early in the project using publicly available NGS sequences (task 2.1).
- Ordered and validated 3 singleplex assays and shared with partner labs (task 2.2)
- WHO mandated only Duoplex assays would achieve EUL approval as new variants emerged.
- Developed a Duoplex SARS CoV-2 assay (task 2.2 re-opened)
- Validated Duoplex assay in various developmental reagents as project progressed (task 2.3)
- AMU generated Armoured RNA standards for an assay (task 2.4)
- Work package completed.

WP3 – progress achievements and results

- All electronics, firmware, software and mechanicals complete for the Alpha (initial prototypes) and are in PCR testing ready to send to partners.
- All design documentation needed for future regulatory approvals are in place.
- Novel instrument and tube completely designed and prototypes made in 12 months.
- Delay in sourcing and receipt of electronic components has delayed the delivery of units (alpha and beta), both due to COVID directly but also the resultant global semiconductor crisis.

WP4 – progress achievements and results

- Designed pan Flu assay In Silico
- Development placed on hold until the COVID assay is finalised – project has given rise to newer generations of buffers, alongside move to Duoplex. As a result it is necessary to focus on the COVID assay and subsequently try and add 2nd target.
- Data has been generated for direct from nasal swab, saliva, gargle samples and from exhaled breath condensates- proof of concept achieved.
- Data generated on lab PCR machines is completed, needs to be repeated on the BGR instrument

WP5 – progress achievements and results

- It has been shown that the method renders SARS CoV-2 non-infectious by the end of the process. (D5.1)
- Partners demonstrated proof of concept early in the project, it was possible to amplify directly from nasal swab eluate (D5.1)
- It was possible to make the reagent as a lyophilized system, performance was equivalent to the frozen reagent (D5.2)
- An assay for the direct detection of Yellow Fever Virus direct from whole human blood developed and validation is ongoing (D5.3)

WP6 – progress achievements and results

- Received ethics approval for the patient sampling
- All partner laboratories have received frozen reagent and the singleplex assay, generated performance report data.
- Proof of concept generated by INMI – it was possible to directly detect from unprocessed nasal swab samples.
- Latterly, all laboratories received Gen 3 frozen buffer and the final Duoplex assay, testing ongoing
- INMI have received the final buffer (Gen 4) and the Duoplex assay and this will be sent to all partners shortly.
- Dried reagent has been evaluated at BGR with equivalent performance to frozen, final shipment of dried Gen 4 (final) buffer was delayed due to the requirement to redevelop from Gen 3.

WP7 – progress achievements and results

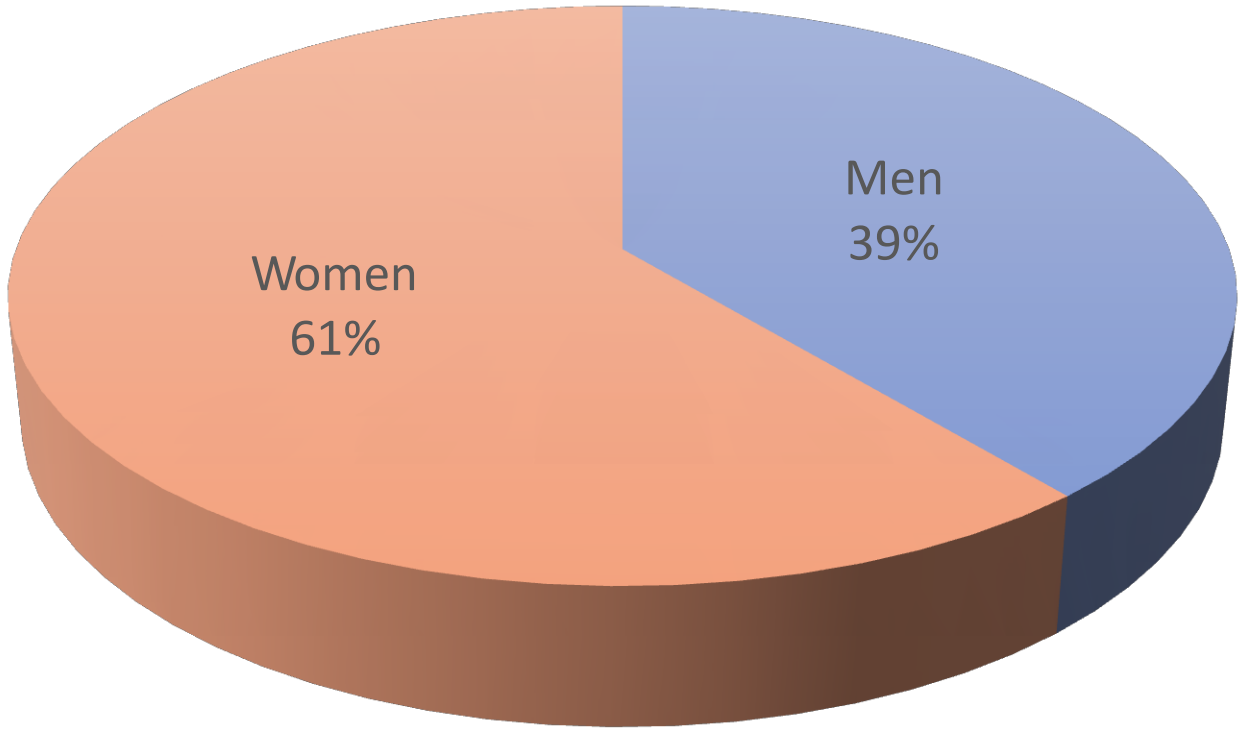
- BGR has filed 2 new patent applications and had a piece of key IP granted during the project
- Knock out and patentability documentation, plus an initial specification has been drawn up for a further filing covering novel aspects of the reagent
- All quarterly reports have been filed

WP8– progress achievements and results

- All required ethics approvals have been put into place.

GENDER

- BGR/BG involve 100% of men (3)
- AMU involve 60% of women (3) and 40% of men (2)
- INMI involve 87,5% of women (7) and 12,5% men (1).
- UCPP involve 50% of women (1) and 50% of men (1)



DATA MANAGEMENT PLAN

Submitted in June 2021

- **Summary of the data** needed to implement the project (purpose, relation to the objectives, types & format, if being re-used, origin, size, utility

Presenting FAIR Data

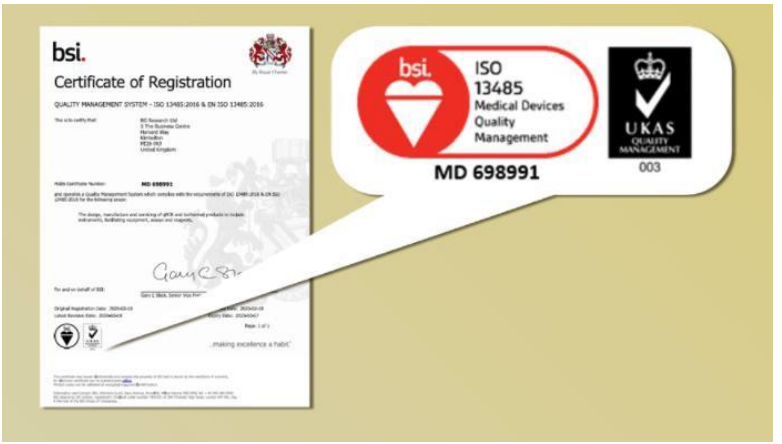
- **Making data findable, including provisions for metadata** (discoverability, identifiability refer to standard identification, convention used, approach toward search keyword, approach for clear versioning, standards for metadata).
- **Making data openly accessible** (open or kept close, how it will be made available, what methods or software tools, where data & metadata are deposited, access/restrictions)
- .**Increase data re-use** (licences, when available for re-use, useable by third parties, quality assurance process, length of time for re-use
- **Allocation of resources** (costs for making data FAIR, responsibilities, potential value for long term preservation
- **Data security** (secure storage)
- **Ethical aspects** (Ethics deliverables)

ISO 13485 QUALITY MANAGEMENT SYSTEM

"The design, manufacture and servicing of qPCR and isothermal products to include instruments, facilitating equipment, assays and reagents"

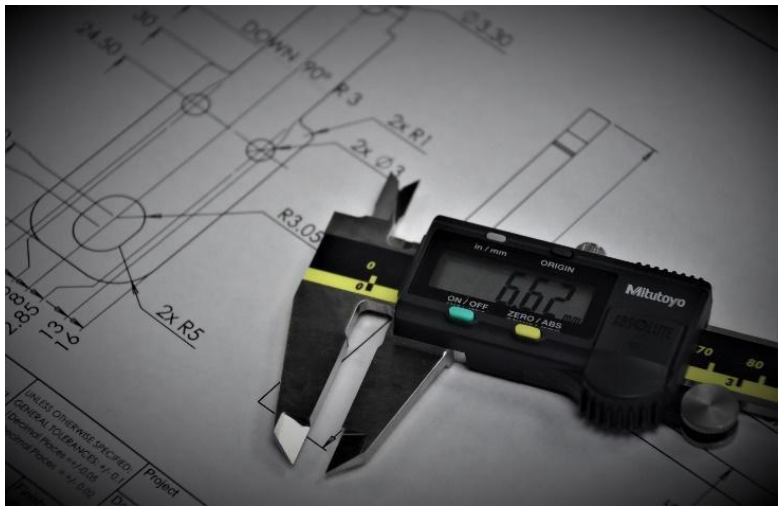
Fully certified (BSI) risk-based ISO 13485 Quality Management System (certified since February 2020)

Management of key documentation (such as requirement specifications, engineering drawings, test reports and standard operating procedures)



Population and collation of project technical files in preparation for regulatory submission

Ongoing auditing of system/site to ensure compliance with ISO 13485:2016 standard and documented procedures



Processing and resolution of non-conformities raised (supplier, internal and customer)

Maintenance of critical business resources and infrastructure (training, calibrations and general maintenance)

DESIGN CONTROLS AND RISK MANAGEMENT

- Key focus on proactive risk management in accordance with ISO 13485:2016 and ISO 14971:2012 standards
- All procedures are based on risk (such as supplier approval and management)
- Regular auditing to identify any potential risks and implement corrective actions (continuous improvement)
- Risk management tools such as Failure Mode and Effects Analysis (FMEA) are utilized to minimise risk to the end user
- Utilization of design controls throughout product development process to ensure end user requirements are successfully met (Design Traceability Matrix and design reviews)
- Gated “waterfall model” used to divide development process up into systematic stages which can easily be managed and controlled

2.0 INSTRUMENT REQUIREMENTS

URS Unique ID	User Requirement	FRS Unique ID	Functional Requirement	Responsibility Assigned To
XF1-URS-2.1	The diagnostic module should be operable in the environments it is likely to be used, from cold, dry air in Europe to hot moist air in Africa [diverse humidity and temperature ranges].	XF1-FRS-2.1A	The diagnostic module must operate in an environment from -10C to 40C.	Nathan Sharp Dave Scott
		XF1-FRS-2.1B	The instrument must operate in humidity ranges from 10-100%.	Nathan Sharp Dave Scott
XF1-URS-2.2	The modular diagnostic system must be capable of performing the diagnostic detection in sub 50 minutes.	XF1-FRS-2.2	The diagnostic module must complete the molecular diagnostic detection/analysis in under 50 minutes.	David Edge

Problems, Risks and Bottlenecks Encountered	Mitigating Action(s)
Alignment of TECs	The sliding method based on aluminium rods and sliding rails had limited accuracy, so the TEC faces were slightly misaligned. When a tube was placed in, the system had to be aligned manually flexing the rig.
Size of Heat sinks	The heat sinks used were 5mm larger than those intended for the final system.
Mounting of Heat Sinks and assembly	The assembly of the system was difficult due to the tolerance in printing with support material and the detail required in the small components. Creating a good slide on the aluminium rods proved challenging.
Wiring drawbacks	The wiring was exposed and at risk of being damaged due to the deliberate mounting in an accessible position. New mounting methods should be designed in future iterations.

Review of Design Results and Ability to Meet Design Input Requirements
The final prototype was capable of thermal cycling tubes according to the thermal cycling process deigned previously. The alpha prototype built was reliable and usable. It preformed the intended function using the specified components and the objectives of the design were met for this stage of design and development.

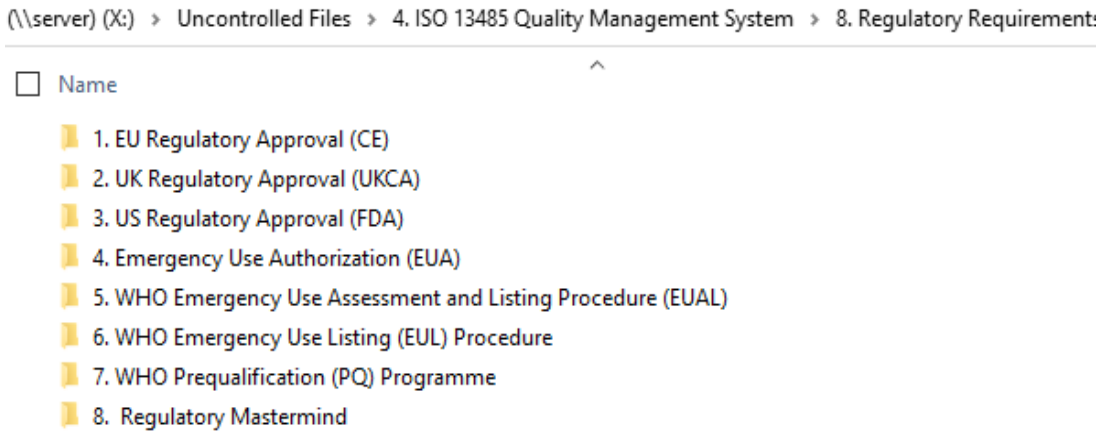
FAILURE MODE AND EFFECTS ANALYSIS (FMEA)

FMEA SUBJECT Optical System

	Item or Process	Potential Failure Mode	Potential Effect(s) of Failure	Sewerity (1-5)	Potential Cause(s) of Failure	Occurrence (1-5)	Current Control(s)	Detection (1-5)	Risk Priority Number (RPN)
F1	Excitation Laser Diodes.	The excitation power could drop from that expected and as a result the observed spectra could fall outside of the instensity ranges expected by the deconvolution algorithm	Reduced light output during overheating event could cause low signal or ultimately false negatives if not rectified	4	Diodes are powered continuously due to electronics failure or heatsink becomes detached, operation outside of specified environmental conditions	1	Two driver chips manage the current being fed to the diodes for redundancy There is a photodiode in the laser diode itself measuring output The environmental operating conditions are clearly stated in the instructions for use	2	8
F2	Excitation laser diodes	The laser diodes could fail entirely and one or other of the excitation sources would no longer function	This could lead to false negatives if not taken into account	4	Component lifetime exceeded or electronic failure either in drivers or the laser diode itself.	2	The current taken by the laser diode is monitored and if the laser diode is not drawing current then the user is warned that the instrument must be serviced before testing can be resumed	2	16

REGULATORY APPROVAL PATHWAYS

- The appropriate regulatory approval must be granted to access markets and sell In Vitro Diagnostic Medical Devices (IVDs)
- Key focus on understanding the regulatory approval pathways and specified requirements
- All key regulatory approval pathways have been researched and guidance documents established detailing the requirements
- Changes in regulation are monitored and analysed on an ongoing basis (such as Brexit and UKCA) by the quality team
- Emergency use authorization regulatory pathways (EUL and EUA) have become a key opportunity since the COVID-19 pandemic
- Applicable regulatory requirements have been communicated to the team to ensure the technical file/product dossier is appropriately prepared for regulatory submission (necessary data and documentation)



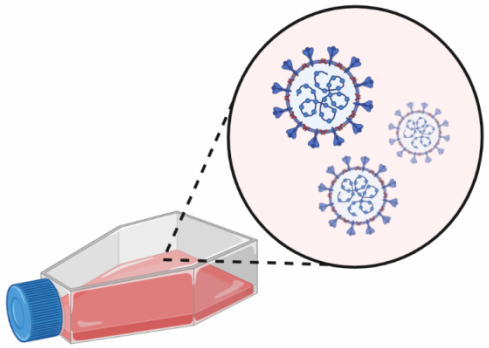
UKCA FDA CE

World Health Organization

MHRA

Verify the inactivation of infectious samples by 30minutes of contact with Master Mix

SARS-CoV-2 strain (Ref-SKU: 026V-03883)
cultured on Vero E6 cells (ATCC#CRL-1586)
(1.4x10^7 -> 1.4x10^-1 TCID50%/mL)

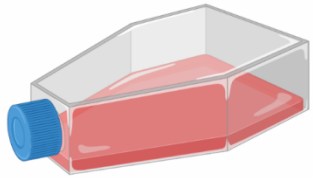


SARS-CoV-2 Gen.3 assay

PCR buffer	1	12,5
Salts	2	0,5
Primers	3	2,25
Probe	4	0,65
Enzyme	5	0,67
Tempalte/Matrix	6/7	5
RTPCR Enhancer	8	0,25
NF Water	NF Water	28,18
Total		45

Contact time: 30 minutes

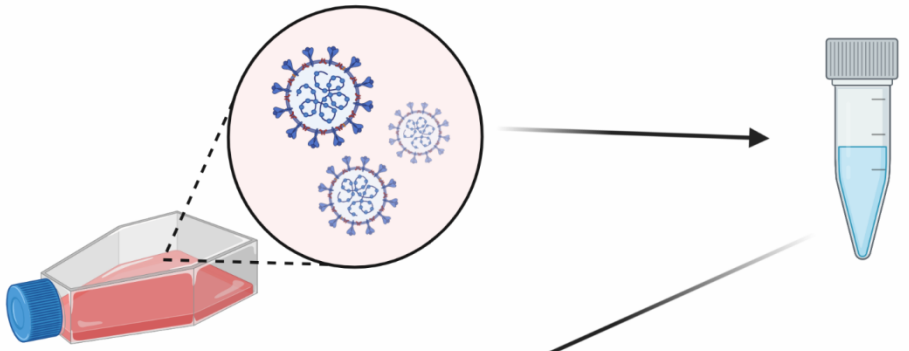
Recultured on
Vero E6 cells



CPE observation
on all samples
after 2x5 days of
culture.

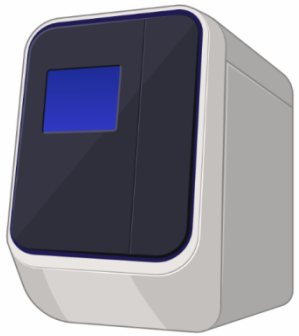
Verify the inactivation of infectious samples after amplifications steps of RT-PCR reactions.

SARS-CoV-2 strain (Ref-SKU: 026V-03883)
cultured on Vero E6 cells (ATCC#CRL-1586)
(1.4x10^7 -> 1.4x10^-1 TCID50%/mL)



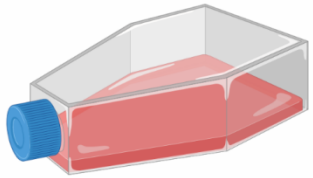
SARS-CoV-2 Gen.2 assay

PCR buffer	1	12,5
Salts	2	0,5
Primers	3	2,25
Probe	4	0,65
Enzyme	5	0,67
Tempalte/Matrix	6/7	5
RTPCR Enhancer	8	0,25
NF Water	NF Water	28,18
Total		45



Cycling		
93°C	10sec	
67°C	120sec	
93°C	4sec	x7 cycles
66°C	60sec	
93°C	10sec	
92°C	4sec	x40 cycles
64°C	30sec	

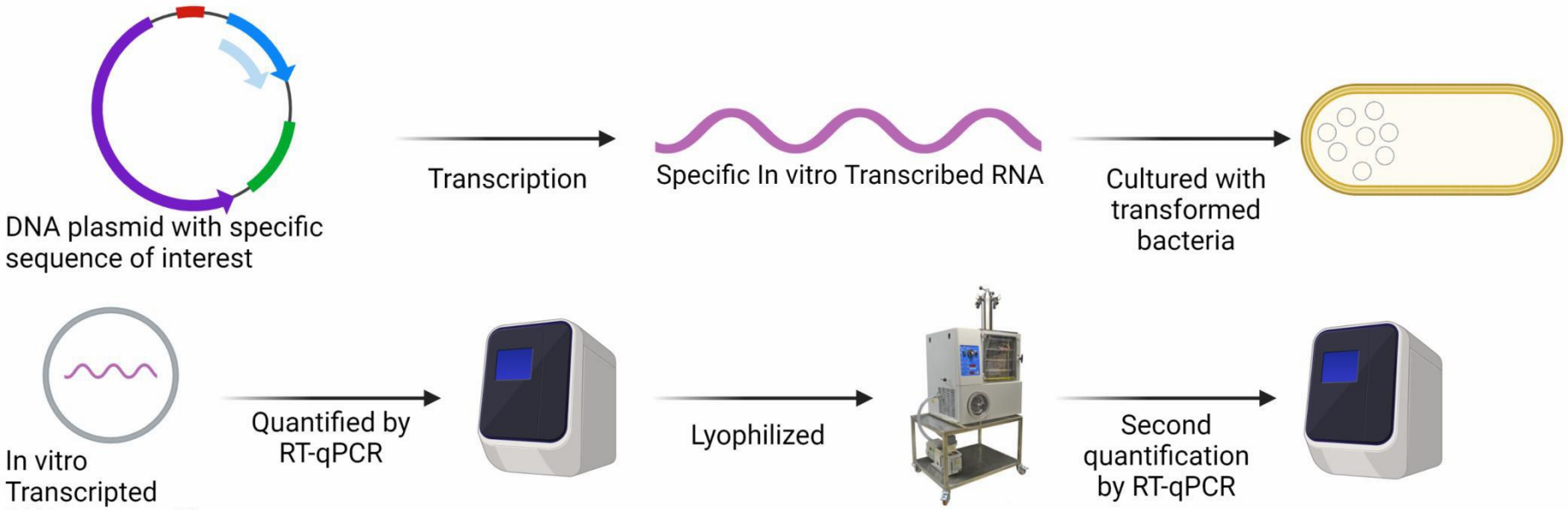
Recultured



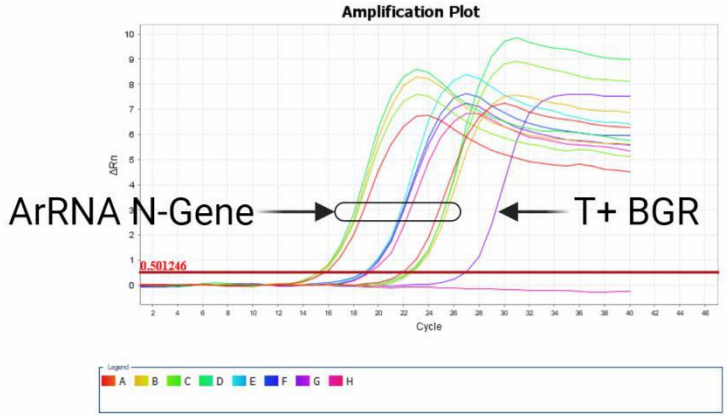
No viral particules detected in PCR products

AMU

Armored RNA assay specific preparation

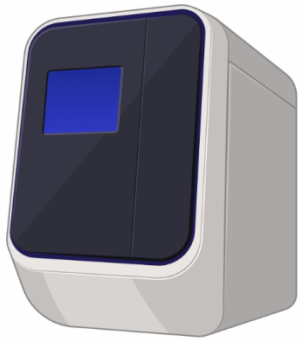


N-gene specific and Yellow Fever specific armored RNA already produced and sent to BGR.

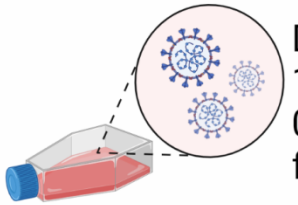


Matrix testing and LoD study of BGR SARS-CoV-2 DUOplex assay

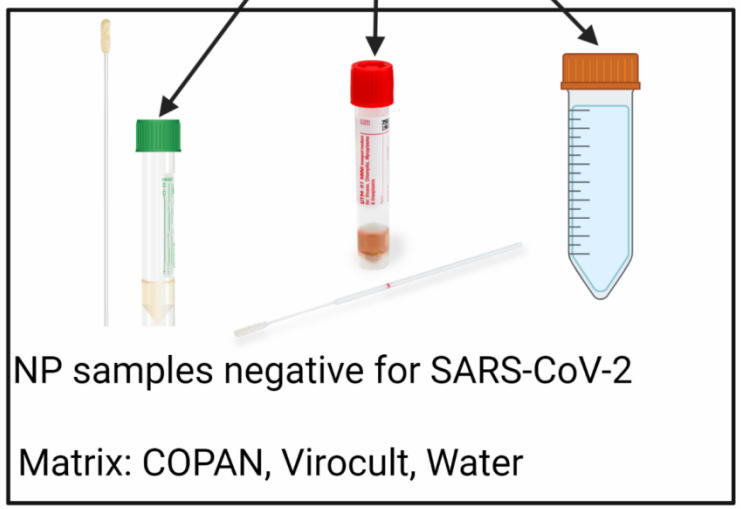
SARS-CoV-2 strain:
- SARS-CoV-2/2020/FR/702 Ref-SKU: 001V-03967
- SARS-CoV-2/2021/FR/7b (lineage B 1. 1 .7)
Ref-SKU: 001V-04044
- SARS-CoV-2/2021/FR/0610 (Lineage B 1.617.2, Delta)
Ref-SKU: 001V-04282



Analyzed by RT-qPCR on
QS 12K Flex and XF-system



Dilution from
10^6 copies/µL to
0.1 copies/µL
for each variant



NP samples negative for SARS-CoV-2

Matrix: COPAN, Virocult, Water

Matrix testing and LoD study of BGR SARS-CoV-2 DUOplex assay

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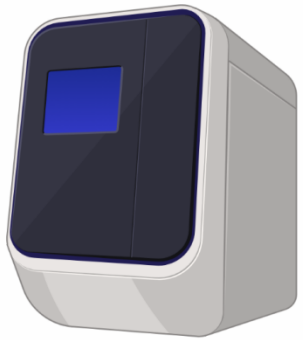

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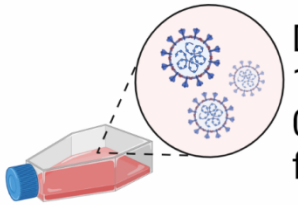

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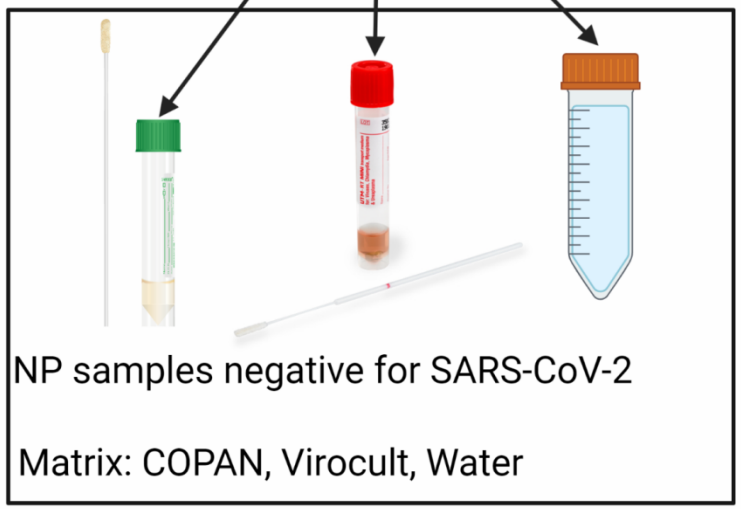

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