SARS-CoV-2 Diagnostic Solutions; inqaba biotec

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Africa's Genomics Company

Dr Aron Abera (Technical Support Manager)
aron.abera@inqababiotec.co.za
www.Inqababiotec.co.za

About inqaba

inqaba biotec™
Africa's Genomics Company

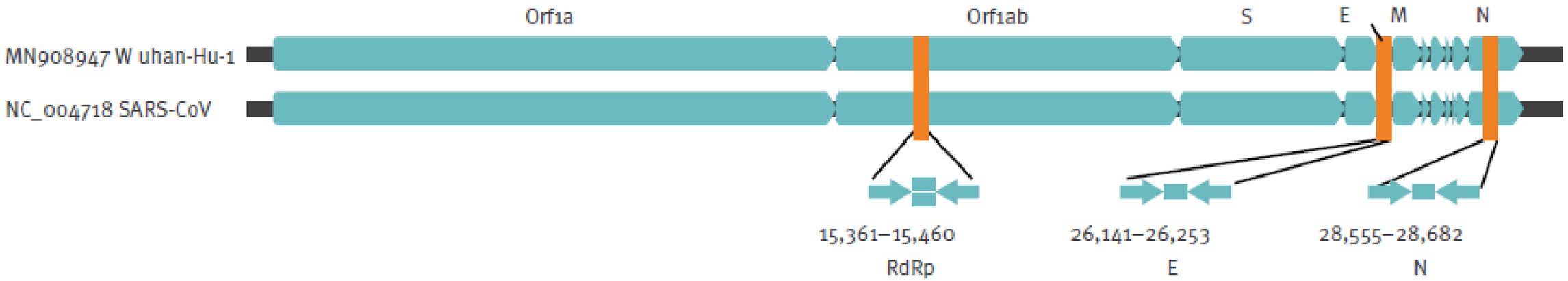
- 2000 — Company Inception
- 2002 — Oligo Production and Sanger Sequencing
- 2007 — NGS Services
- 2010 — East Africa Subsidiary
- 2014 — West Africa Subsidiary
- 2016 — Eppendorf
- 2017 — Pac Bio

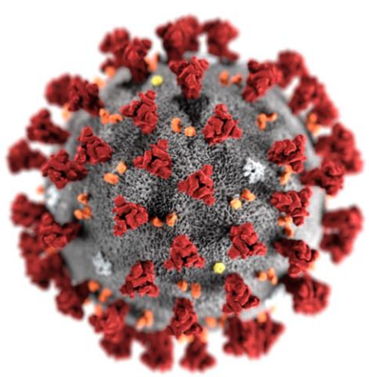
SARS-CoV-2

Severe **A**cute **R**espiratory **S**yndrome **Co**rona**V**irus **2**

- RNA Virus
- Cause of COVID-19 disease

Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome





Corman et al 2020

Approved Workflow of SARS-CoV2 Molecular Diagnosis

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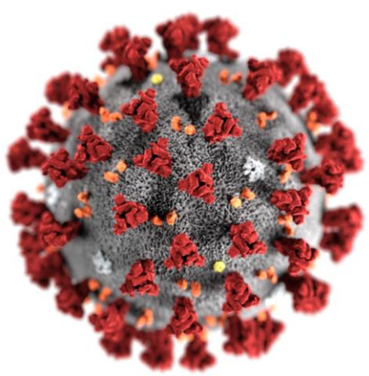
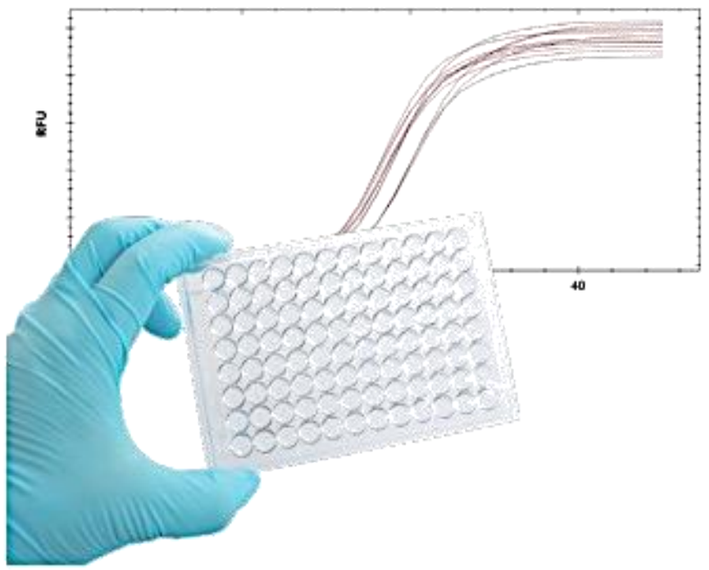
Collect Specimens

Extract RNA





Analysis

Specimen Collection

Challenges

- Safety regarding specimen collection, handling & transportation

Solution

- DNA/RNA Shield™ Collection Devices from Zymo
- Collect specimens using DNA/RNA Shield™ Collection Devices
- SARS-CoV-2 RNA is stable in DNA/RNA Shield™ for **28 days** at room temperature
- Samples in DNA/RNA Shield™ can be shipped at ambient temperature – no cold chain needed

RNA Extraction

Challenges

- Low throughput RNA extraction and RNA extraction not optimized for biological fluids/ clinical samples

Solution

- RNA Extraction and Purification Quick-DNA/RNA Viral MagBead Kit from Zymo Research
  - Automated and Manual RNA extraction
  - Optimized for Biological fluids (Upper and lower respiratory specimen)
  - Compatible with UTM/VTM, Saline, PBS and liquid amies
- Monarch Total RNA Miniprep kit

RNA Extraction (Automation)

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Primers and Probes

- Custom primer and probes for SARS-CoV-2 qPCR detection synthesized locally by inqaba biotec
  - Primers and probes synthesized with speed, accuracy and purity
  - Compatible probes synthesized for multiplexing serval targets of SARS-CoV-2 and human internal control (e.g. FAM, HEX and others)

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Facilitating Detection of SARS-CoV-2 Individual Reagents

RNA detection Reagents (NEB)

Challenges

- The inclusion of an up-front RNA purification step, adds to protocol time and cost per sample, and overall can reduce testing throughput

Solution

- Luna Universal Probe One-Step RT-qPCR kit
  - Hydrolysis probe-based detection of RNA

- LunaScript RT SuperMix kit
  - First strand synthesis in two-step RT-qPCR workflows

- WarmStart Colorimetric LAMP Master mix
  - For rapid reverse transcription and isothermal amplification of RNA targets, with a simple visible detection

Sequencing and COVID-19 (NEB)

- Illumina Sequencing (Reagents and Services)
  - Using the ARTIC SARS-CoV-2 primers (400bp) sample pooling and sequencing on the Illumina
    - Q5® Hot Start High-Fidelity DNA Polymerase
    - NEBNext® Ultra™ II End Repair / dA-Tailing Module
    - NEBNext Ultra II Ligation Module
    - NEBNext Quick Ligation Module

- PacBio HiFi Sequencing (Reagents and Services)
  - Long amplicon virus sequencing

- Nanopore Sequencing
  - Amplicon sequencing protocol for MinION for nCoV-2019,
    - Q5® Hot Start High-Fidelity DNA Polymerase
    - NEBNext® Ultra™ II End Repair / dA-Tailing Module
    - NEBNext Ultra II Ligation Module
    - NEBNext Quick Ligation Module

Allplex SARS-CoV-2 Assay (Seegene)

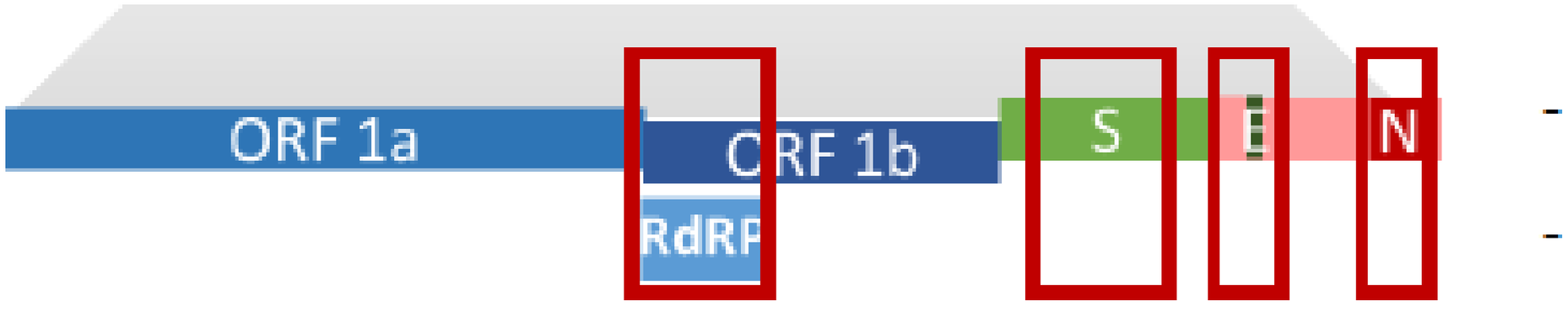
- Seegene, a company with wide range of solutions applicable in diverse markets from clinical diagnostics to basic science research

  - Auto extraction System
    - Seegene STARlet/Nimbus
    - SEEPREP32
    - NucliSENS eayMAG

  - Real Time PCR
    - CFX96 SW IVD v1.6 and Dx System v3.1
    - AB 7500 SW v2.05

Allplex SARS-CoV-2 Assay (Seegene)

- Four target genes assay
- Testing principle Real time PCR
  - Multiplex Real-time PCR assay to detect 4 targets (RdRP, S and N gene specific for SARS CoV-2 and E gene for all of Sarbecovirus including SARS-CoV-2
  - Exogenous gene is used as an internal control (IC) to monitor the whole process.



Allplex SARS-CoV-2 Assay (Seegene)

- Sample Types
  - Nasopharyngeal/throat swab, Nasopharyngeal aspirate bronchoalveolar lavage and sputum
- Analytical Sensitivity
  - 50 copies/rxn for all targets
- Cut-offs
  - All analytes: ≤40 cycle
  - IC: ≤40 cycle

Allplex SARS-CoV-2 Assay (Seegene)

- Supplies and Accessories needed
  - RNA stabilization media
  - RNA extraction kit if not using NIMBUS automated extraction kit
  - Laboratory Consumables (Gloves, pipettes, plates, tips …)

- Contents
  - Oligo Mix
  - PREMIX
  - Control (SARS2 PC
  - Control IC
  - Rnase-free water

Test kits (50 and 100 reactions) and should be stored at -20° C

Allplex SARS-CoV-2 Assay (Seegene)

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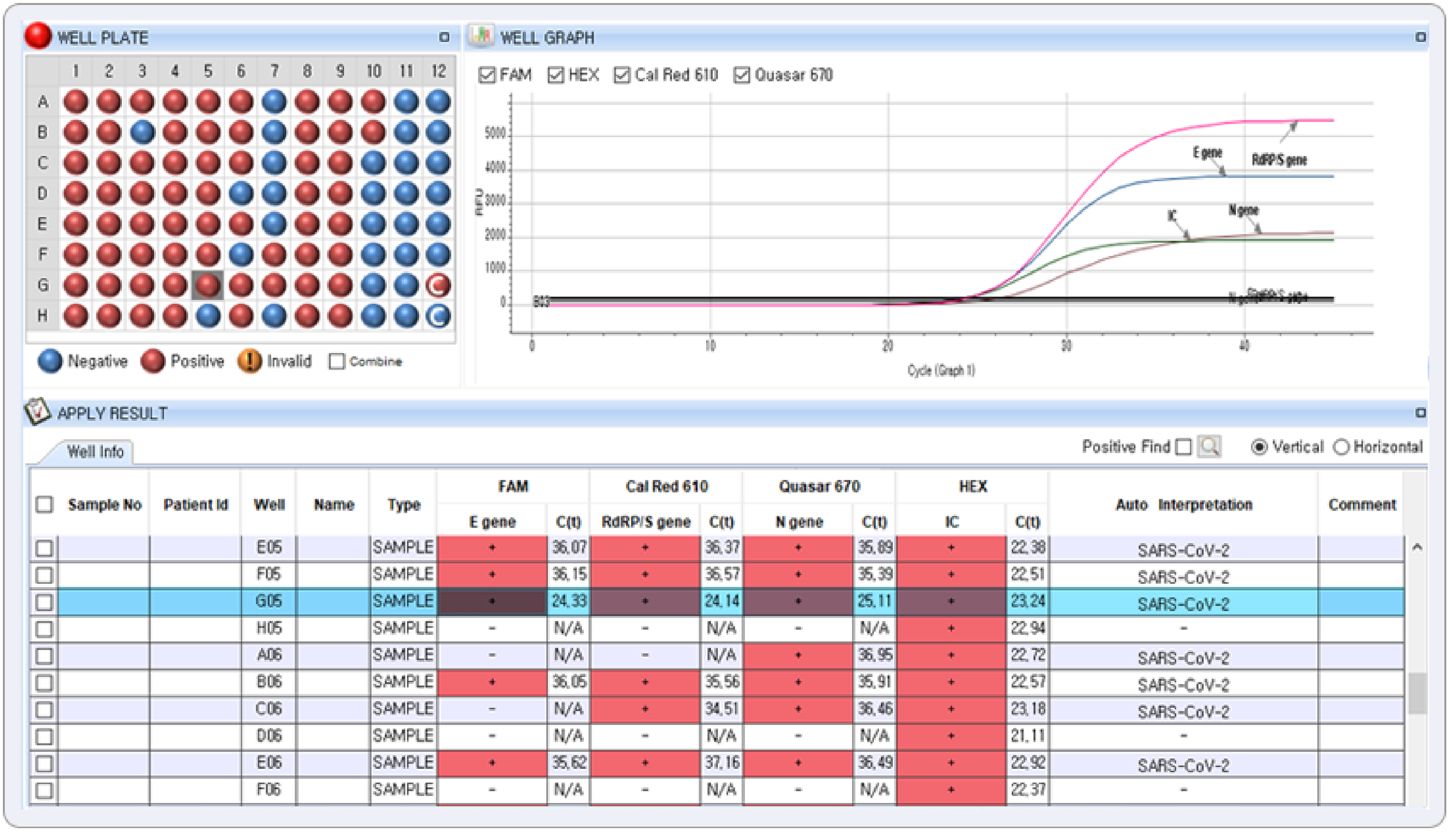
Interpretation of Results

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
IC (HEX)	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+	-
E gene (FAM)	+	+	-	+	-	-	+	-	-
RdRP gene / S gene (Cal Red 610)	+	-	+	+	+	-	-	-	-
N gene (Quasar 670)	+	+	+	-	-	+	-	-	-
Result Interpretation	**SARS-CoV-2 Detected**						Presumptive positive	**Negative**	**Invalid**

Allplex SARS-CoV-2 Assay (Seegene)



Sample No	Patient Id	Well	Name	Type	FAM		Cal Red 610		Quasar 670		HEX		Auto Interpretation	Comment
					E gene	C(t)	RdRP/S gene	C(t)	N gene	C(t)	IC	C(t)		
		E05		SAMPLE	+	36.07	+	36.37	+	35.89	+	22.38	SARS-CoV-2	
		F05		SAMPLE	+	36.15	+	36.57	+	35.39	+	22.51	SARS-CoV-2	
		G05		SAMPLE	+	24.33	+	24.14	+	25.11	+	23.24	SARS-CoV-2	
		H05		SAMPLE	-	N/A	-	N/A	-	N/A	+	22.94	-	
		A06		SAMPLE	-	N/A	-	N/A	+	36.95	+	22.72	SARS-CoV-2	
		B06		SAMPLE	+	36.05	+	35.56	+	35.91	+	22.57	SARS-CoV-2	
		C06		SAMPLE	-	N/A	+	34.51	+	36.46	+	23.18	SARS-CoV-2	
		D06		SAMPLE	-	N/A	-	N/A	-	N/A	+	21.11	-	
		E06		SAMPLE	+	35.62	+	37.16	+	36.49	+	22.92	SARS-CoV-2	
		F06		SAMPLE	-	N/A	-	N/A	-	N/A	+	22.37	-	

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THANK YOU.