STILLA

CRYSTAL DIGITAL PCR™ DETECTION KIT FOR SARS-COV-2

Romain Parillaud, PhD
Field Application Specialist
Stilla Technologies Inc.

Current molecular diagnostic tool to diagnose SARS-CoV-2

- Clinical symptoms difficult to dissociate from other respiratory infections
- Detection of SARS-CoV-2 Nucleic acid sequence

Real-Time PCR

- Patient having pneumonia and CT abnormalities but be initially RT-qPCR negative for SARS-CoV-2
  => Only later after onset observed positive on RT-PCR

Ai T, et al. Radiology, 2020.
Kanne JP, et al. Radiology, 2020
Yang W, et al. Radiology, 2020.
Winichakoon P, et.al. Journal of Clinical Microbiology Feb 2020

- RT-qPCR reported to be 50-70% sensitive

Arima Y,Emerg Infect Dis.2020
Wu J, et al. Clin Infect Dis 2020
Fengting Yu, et al. Clin Infect Dis. 2020
Xie X, et.al. Radiology. 2020

Necessity to develop more sensitive detection tools

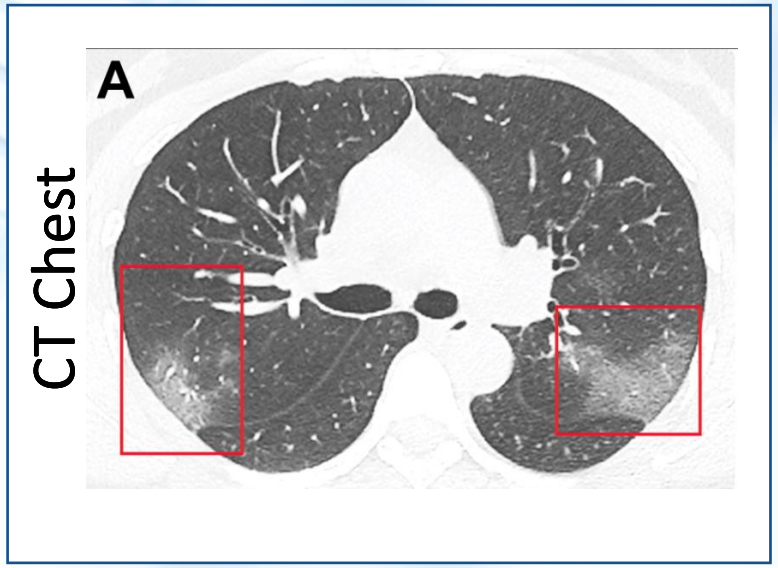
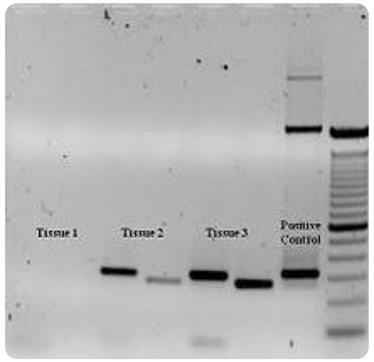


Image: https://www.itnonline.com/article/ct-imaging-2019-novel-coronavirus-2019-ncov-pneumonia

NEXT GENERATION OF PCR
Digital PCR

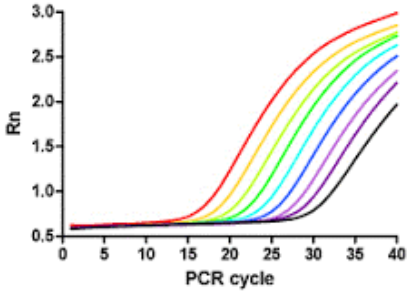
OUR MISSION:
MAKE DIGITAL PCR A LAB COMMODITY

PCR



Amplify Target DNA

Quantitative PCR

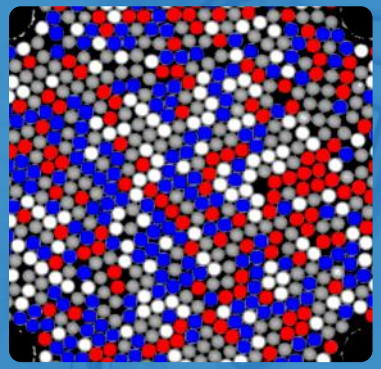


Relative quantification

Real-time with standard curves

Ubiquitously spread method

Digital PCR



Absolute quantification

No standard curve
Increased sensitivity

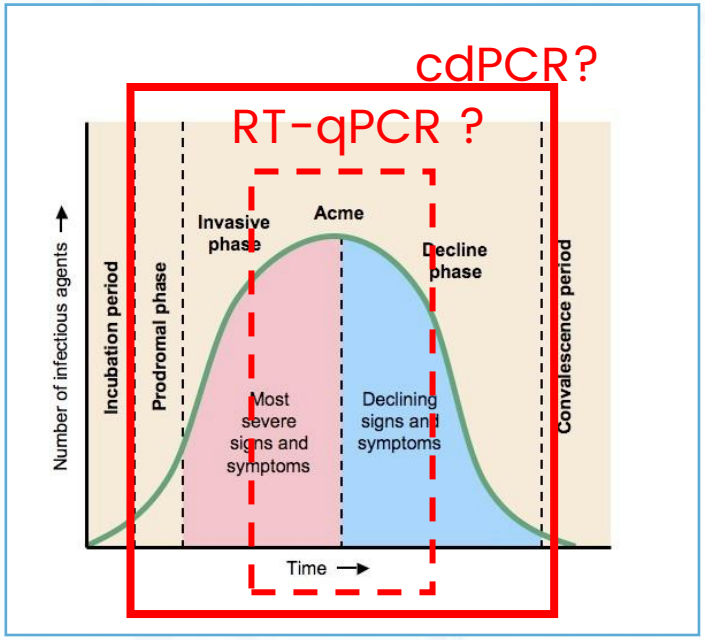
Higher precision

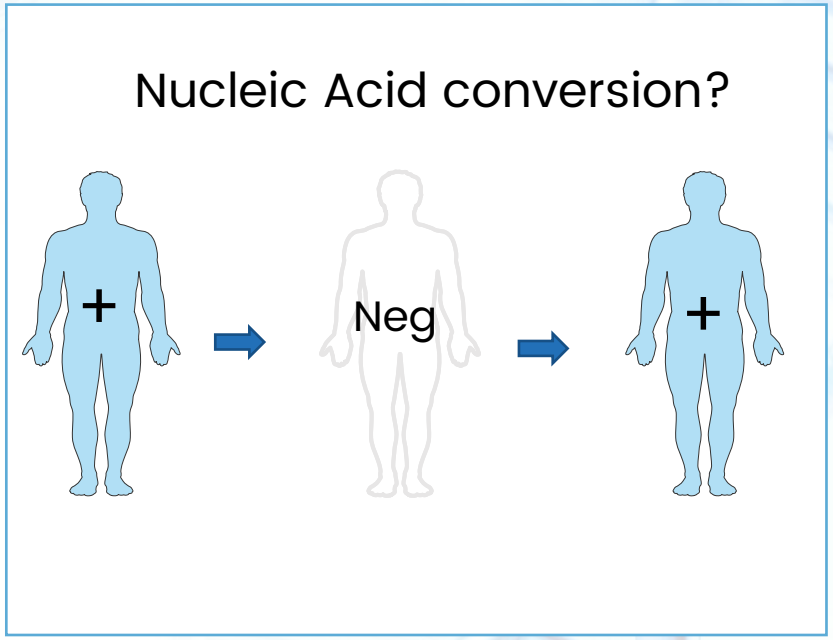
NEXT GENERATION OF PCR
Digital PCR

OUR MISSION:
MAKE DIGITAL PCR A LAB COMMODITY

- Digital PCR could be a valuable asset in the COVID-19 battle

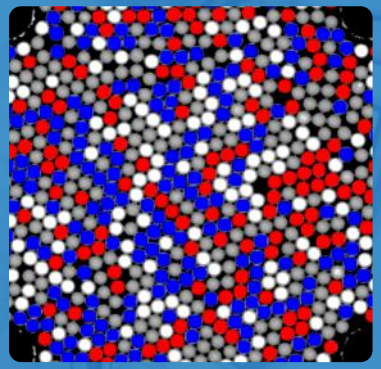
Sensitivity





Ai Tang Xiao, Yi Xin Tong, Sheng Zhang, , J Med Virol. 2020 Apr 9

Digital PCR



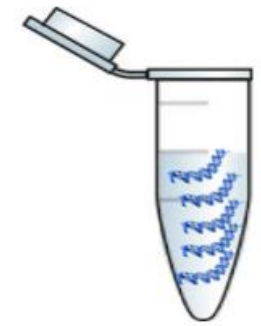
Absolute quantification

No standard curve
Increased sensitivity

Higher precision

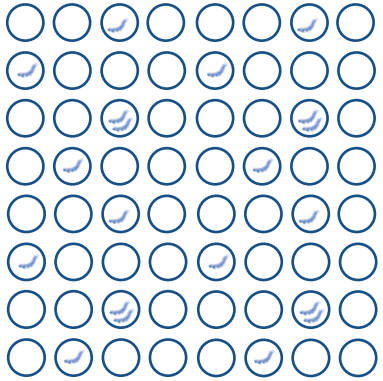
PRINCIPLE
OF DIGITAL PCR

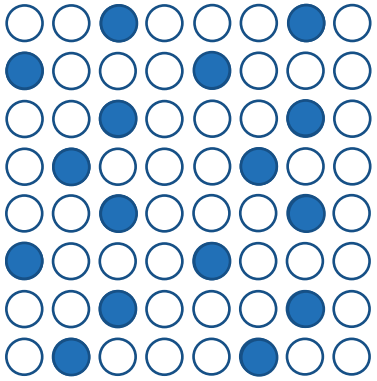
PARTITIONING



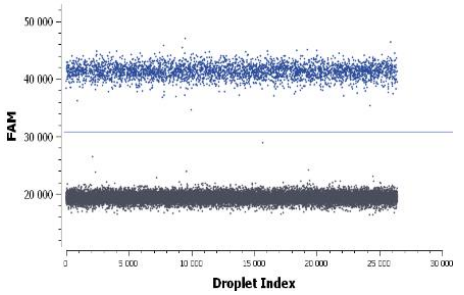
Wild-type DNA

PCR





READING & ANALYSIS



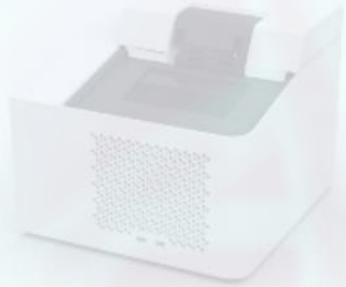
RESULTS
2636 cp/µL with **2.2 %** uncertainty

POISSON STATISTICS

$$\frac{N_{pos}}{N_{tot}}$$

Naica™ System Workflow
Crystal Digital PCR™

Sapphire Chip
(consumable)



Sapphire Chip : pre-filled with oil

Input volume 25 µL

› **patented partitioning technology: droplet crystals.**

Droplets per sample ~ 30 000

Droplet volume 0.59 nL

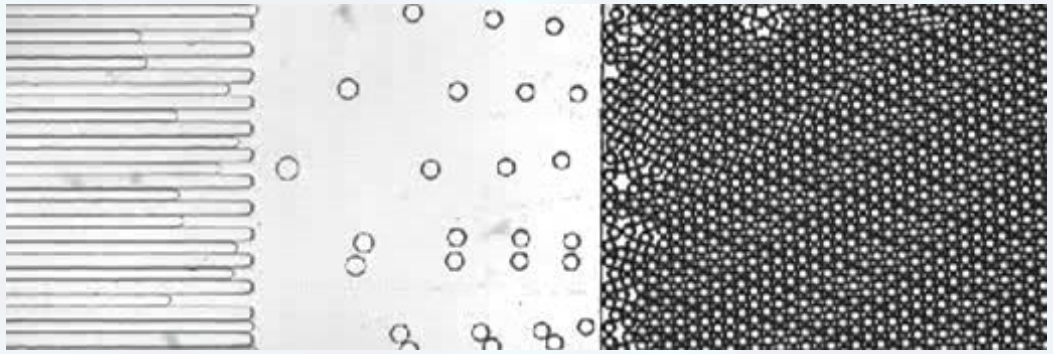
Naica™ System Workflow
Crystal Digital PCR™

Sapphire Chip
(consumable)

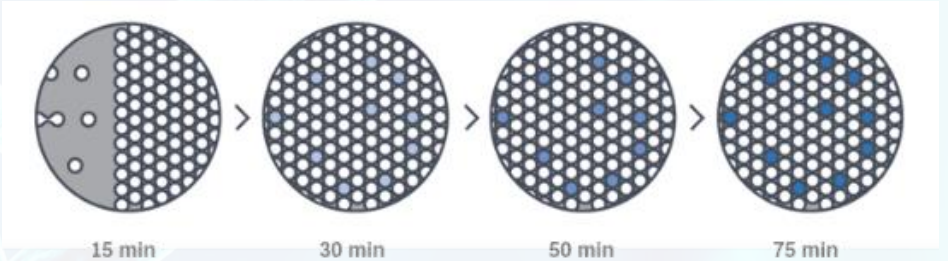
Naica™ Geode

Step 2.1 – Partition

- 1-3 chips and 1-12 samples / run
- Contactless fluid injection



Step 2.2 – Amplify

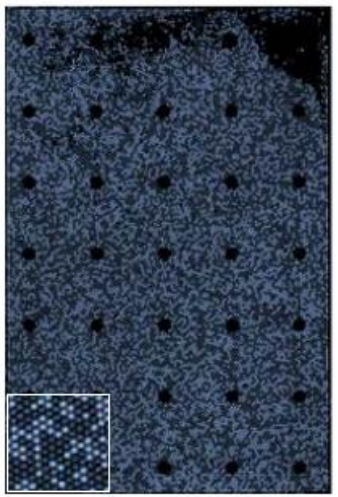


Naica™ System Workflow
Crystal Digital PCR™

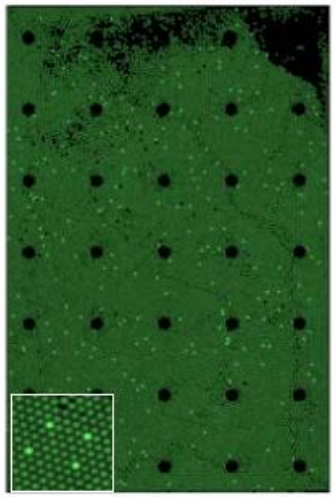
Sapphire Chip
(consumable)

Naica™ Geode

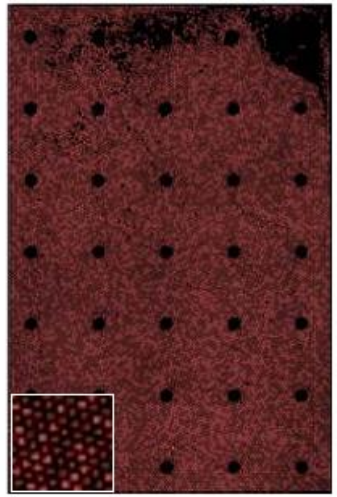
Naica™ Prism3



Blue
Ex: 415-480 nm
Em: 495-520 nm
FAM…



Green
Ex: 530-550 nm
Em: 560-610 nm
HEX…



Red
Ex: 615-645 nm
Em: 655-720 nm
Cy®5…

Naica™ System Workflow

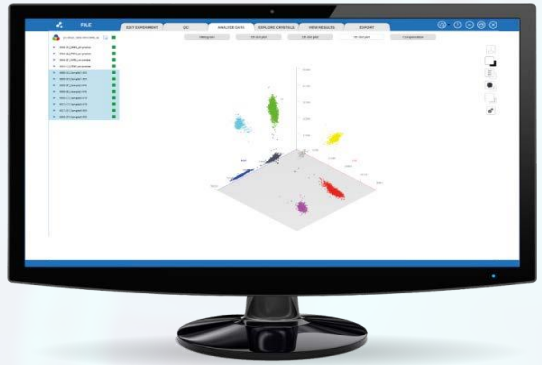
Crystal Digital PCR™

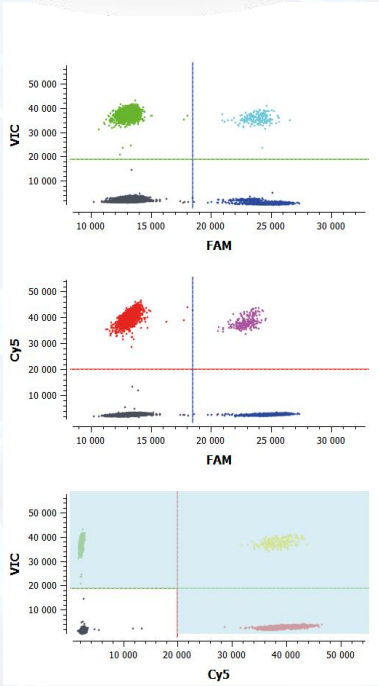
Sapphire Chip (consumable)

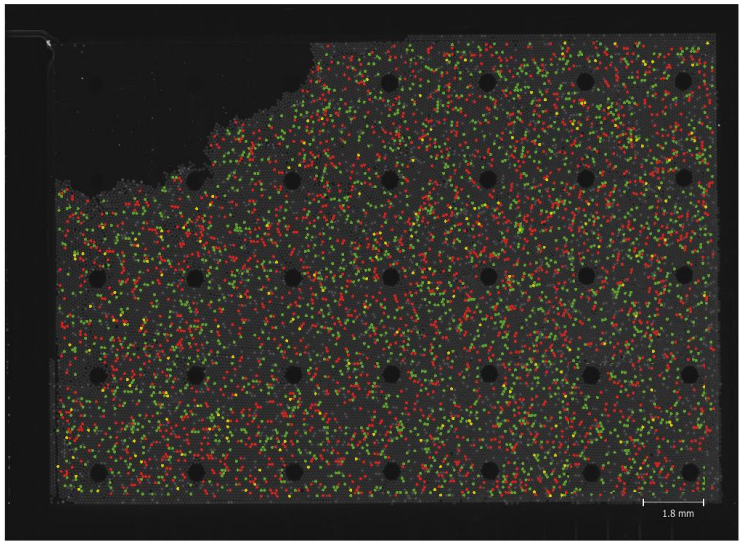
Naica™ Geode

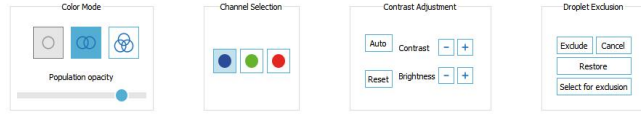
Naica™ Prism3

Crystal Miner™ (software)



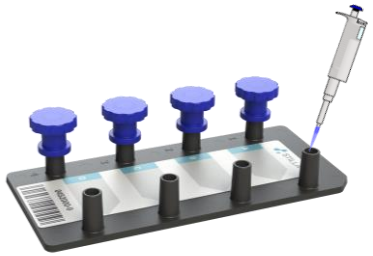


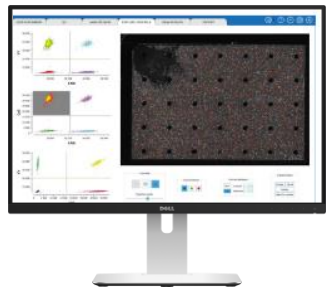




PERFORM CRYSTAL DIGITAL PCR™

IN 2H30 WITH MINIMUM HANDS-ON TIME





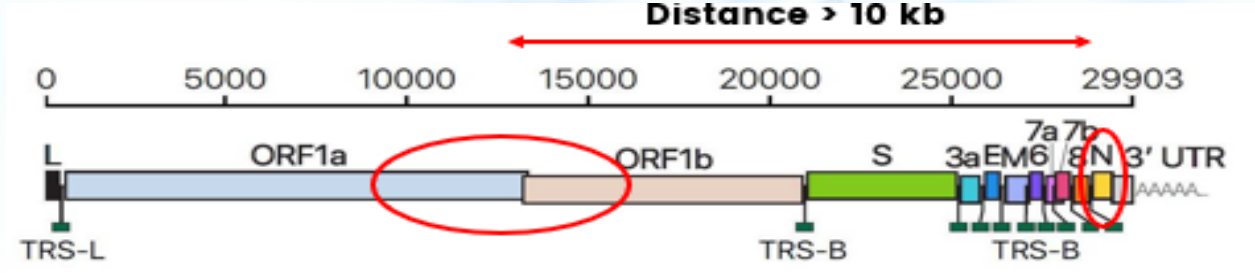
DESCRIPTION	Pipette 25 µL of PCR mix into the Sapphire Chips and seal with cap	Place Sapphire chip into the Geode and launch the combined partitioning and thermocycling program	Image Chips using three fluorescent detection channels	Analyze results using our intuitive Crystal Miner software
PROCESS TIME 2H30	**5 min**	**2h10 min**	**10 min**	**5 min**
HANDS-ON TIME 5 min	**5 min**	**15 s**	**15 s**	

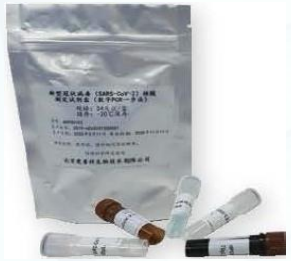
A novel kit for COVID-19 detection in human samples

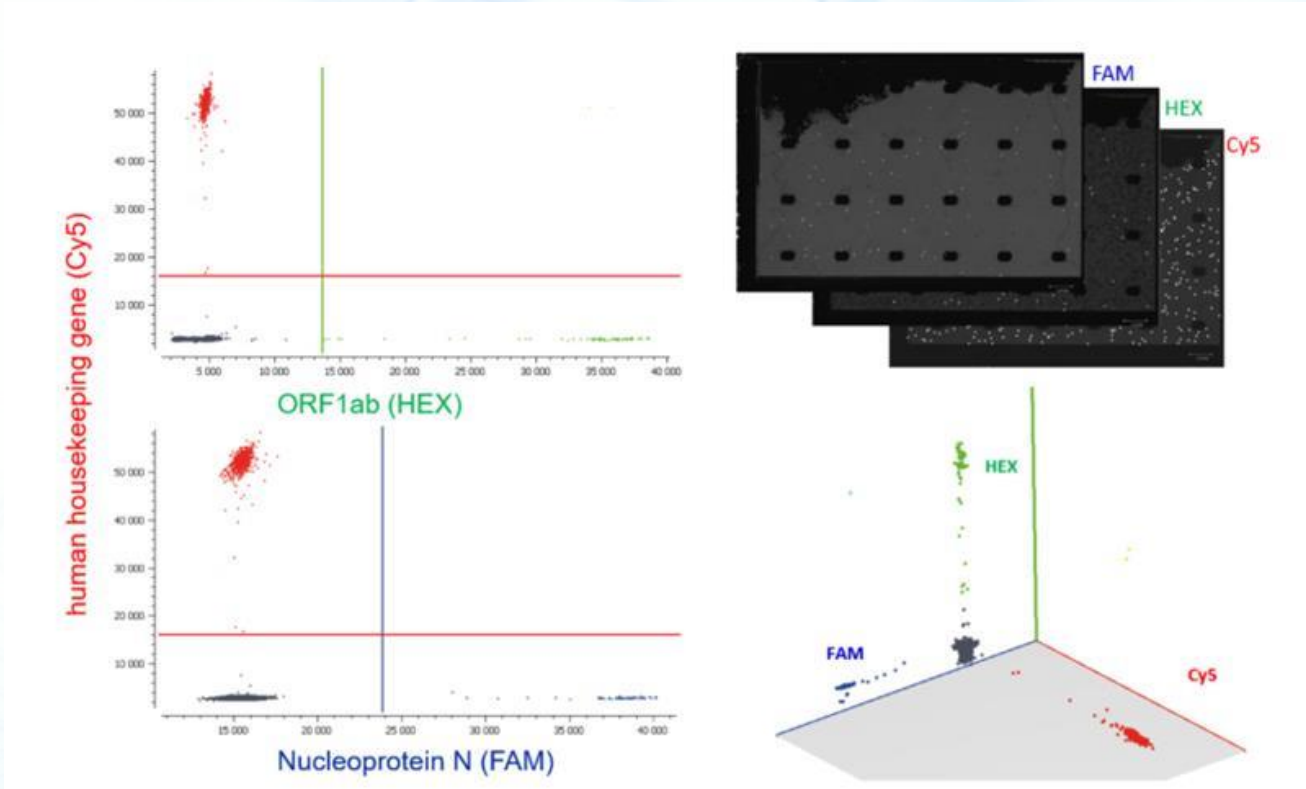
Taking advantage of multiplexing for increase sensitivity

3-color kit to detect viral and human genes:

- COVID-19 ORF1ab (HEX)
- COVID-19 nucleoprotein N (FAM)
- Human control housekeeping gene (Cy5)





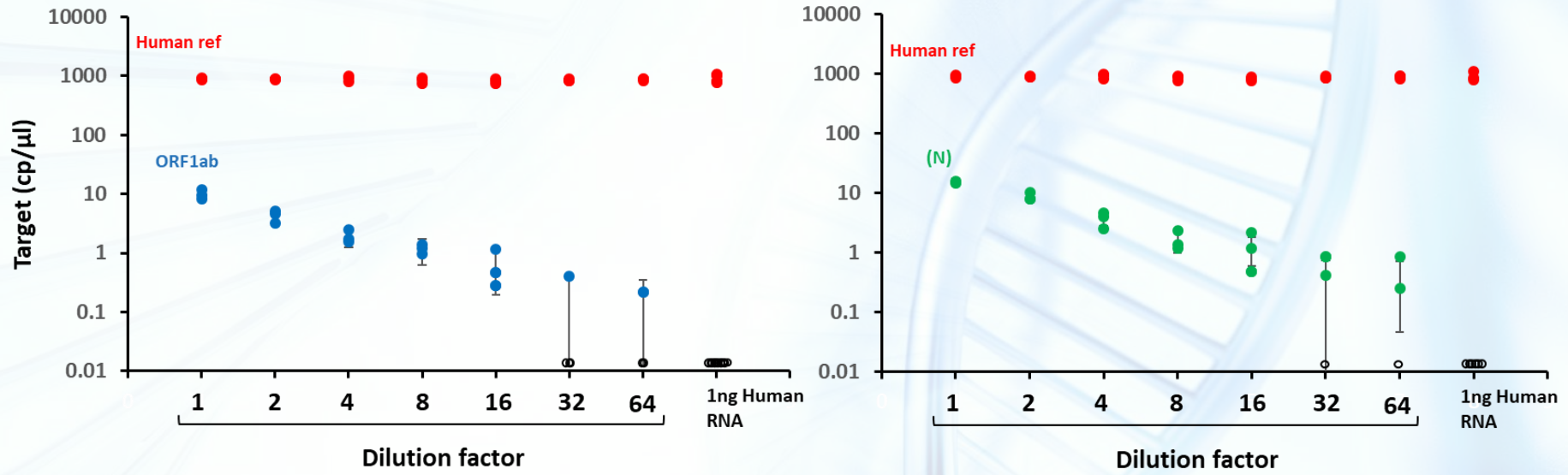


Kit components

- dPCR master mix1
- dPCR master mix2
- Primer and probe mix
- COVID-19 positive control
- COVID-19 negative control
- Sapphire Chips

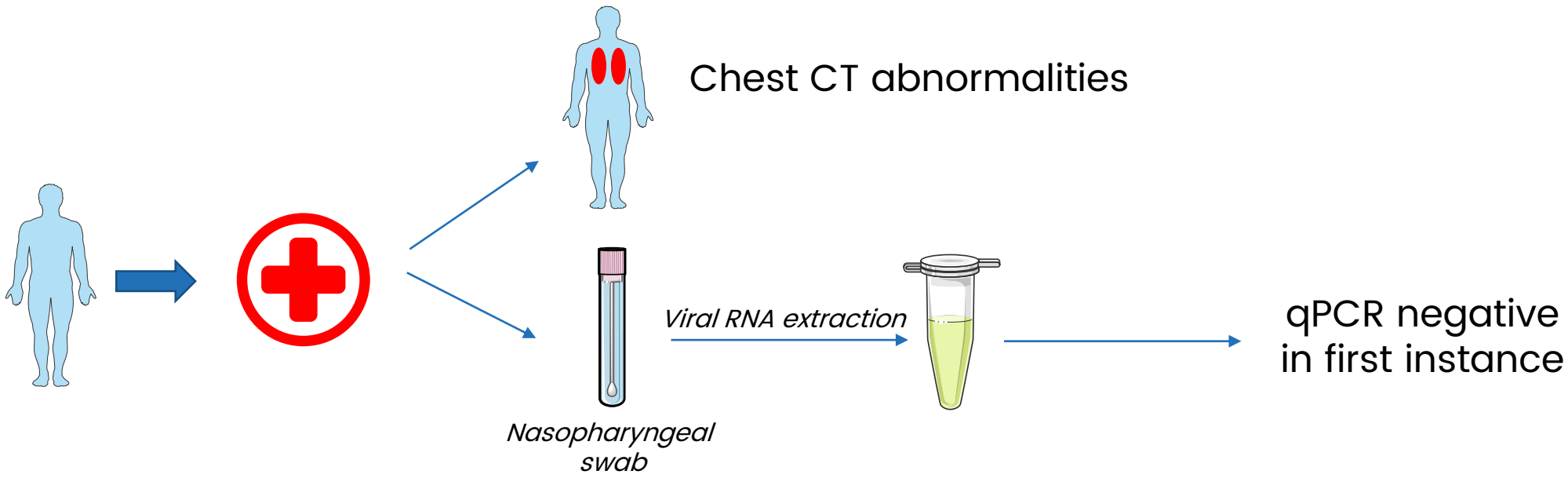
Sensitive and specific detection of COVID-19 sequences

- A positive control containing ORF1ab and nucleoprotein N sequences was serially diluted and tested in triplicate.
- A total of 8 µl of positive control was assessed in a 25µl reaction in a background of 1 ng of human RNA.



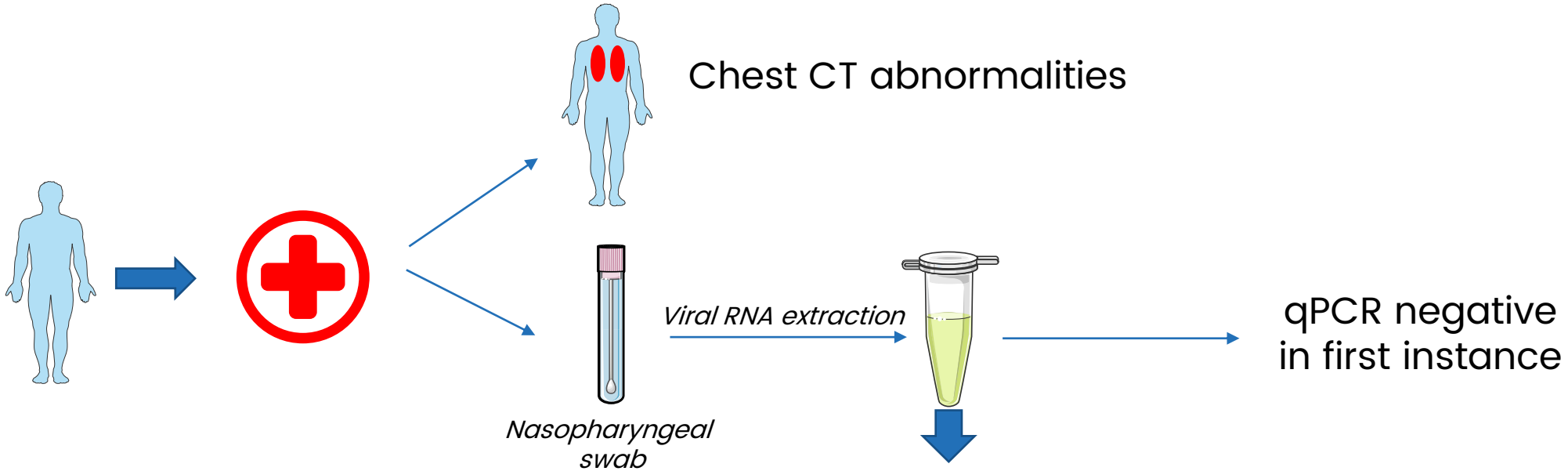
- The Crystal Digital PCR kit for COVID-19 detection was shown to reliably identify the viral sequences
  - ORF1ab: down to 5 copies per 25µl reaction (equivalent to 0.2 cp/µl)
  - Nucleoprotein N: down to 7 copies per 25µl reaction (equivalent to 0.28 cp/µl)
- No false positives were observed in 15 negative controls containing 1 ng of human RNA per 25 µl reaction

Preliminary results
Investigation of CT+/qPCR- patients



 Data obtained in collaboration with virology and genetics departments (V. Thibault, C. Grolhier, MD. Galibert and A. Lespagnol)

Preliminary results
Investigation of CT+/qPCR- patients



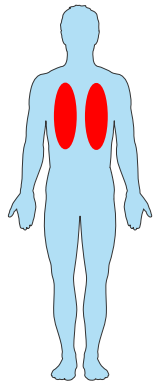
Naica System

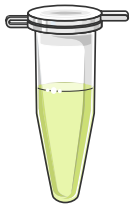
COVID-19
Detection kit

 Data obtained in collaboration with virology and genetics departments (V. Thibault, C. Grolhier, MD. Galibert and A. Lespagnol)

Preliminary results
Investigation of CT+/qPCR- patients

10 patients





Viral RNA extraction

qPCR negative
in first instance

Patient #	Viral charge in Crystal Digital PCR
Patient 1	negative
Patient 2	**positive**
Patient 3	**positive**
Patient 4	negative
Patient 5	negative
Patient 6	negative
Patient 7	negative
Patient 8	**positive**
Patient 9	**positive**
Patient 10	negative

4 patients on 10 were retrieved positives

Could bring relevant clinical information at the first onset of the infection

Data obtained in collaboration with virology and genetics departments (V. Thibault, C. Grolhier, MD. Galibert and A. Lespagnol)

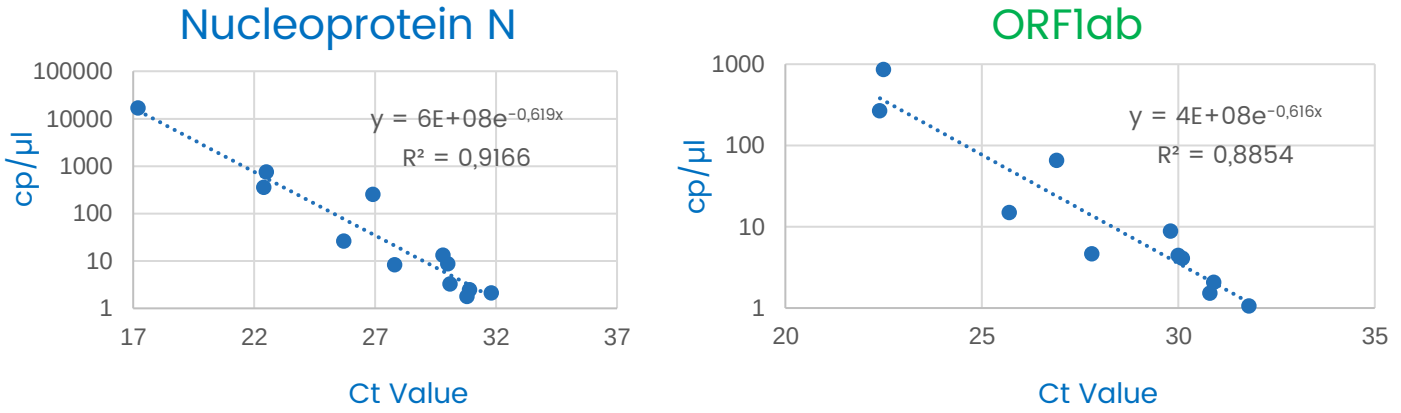
COVID-19 quantification by cdPCR

- Patient samples originally tested by qPCR were evaluated with cdPCR
  - Investigate high Ct values obtained by qPCR

qPCR	Number of patient tested
Negative ***(Undetermined)***	**15**
Positive (Ct<35)	**12**
Doubtful (Ct>34)	**18**

- One recall, determined positive by cdPCR

Correlation cdPCR (cp/µl) and qPCR (Ct) on positive samples



Full correlation cannot be reach, as qPCR is not standardized and patient samples are collected and extracted from different series

Data obtained in collaboration with Pr. Drancourt & Dr. Bouam at Unité MEPHI, IHU Méditerranée Infection, Marseille.

COVID-19 quantification by cdPCR in high Ct qPCR data

qPCR	Number of patient tested
Negative ***(Undetermined)***	**15**
Positive (Ct<35)	**12**
Doubtful (Ct>34)	**18**

Numéro	CT qPCR	Viral charge determined in cdPCR
1	37.32	neg
2	35.53	pos
3	35.15	pos
4	34.65	neg
5	36.92	neg
6	36.56	neg
7	36.75	pos
8	35.17	Pos
9	34.37	pos
10	37.43	pos
11	34.95	pos
12	38.17	neg*
13	33.78	pos
14	36,35	pos
15	36,66	neg
16	34,3	pos
17	36,96	neg
18	36,47	pos

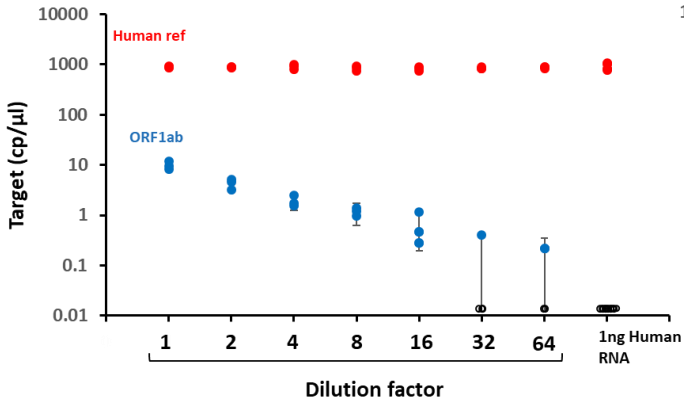
**IC highlight a possible Collection/Extraction issue*

Viral absence confirmed by cdPCR	**7/18**
Viral presence confirmed by cdPCR	**11/18**

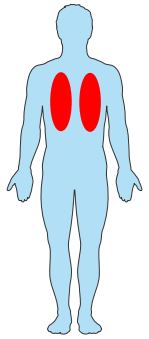
Crystal Digtial PCR Covid-19 detection kit is a easy and fast solution to investigate difficult to interpret high Ct value qPCR data.

Data obtained in collaboration with Pr. Drancourt & Dr. Bouam at Unité MEPHI, IHU Méditerranée Infection, Marseille.

Conclusion



10 patients



qPCR negative in first instance

4/10 Positives

High qPCR Ct values

Viral absence confirmed by cdPCR	7/18
Viral presence confirmed by cdPCR	11/18

- **Crystal Digital PCR™ Covid-19 detection kit can detect only few copies of the SARS-CoV2**
- **Could bring relevant clinical information at the first onset of the infection**
- **Crystal Digital PCR Covid-19 detection kit is a easy and fast solution to investigate difficult to interpret high Ct value qPCR data.**

STILLA

SPECIAL THANK YOU TO:

Pr. M.Drancourt
Dr. A.Bouam

Dr. V.Thibault
Dr. C.Grolhier
Dr. M.D.Galibert
Dr. A.Lespagnol

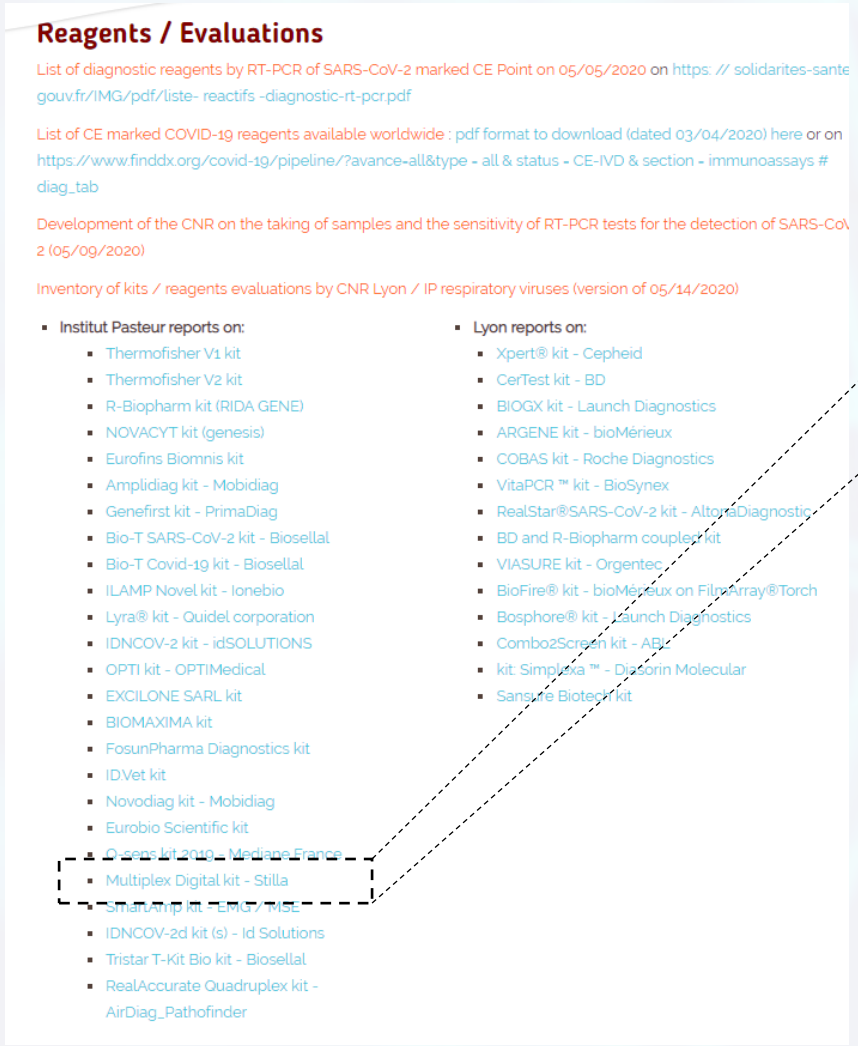
THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

For more information on product and workflow, visit our website at

www.stillatechnologies.com

Evaluation of Crystal Digital PCR COVID-19 detection kit by the Institut Pasteur



Reagents / Evaluations

List of diagnostic reagents by RT-PCR of SARS-CoV-2 marked CE Point on 05/05/2020 on https: // solidarites-sante gouv.fr/IMG/pdf/liste- reactifs -diagnostic-rt-pcr.pdf

List of CE marked COVID-19 reagents available worldwide : pdf format to download (dated 03/04/2020) here or on https://www.finddx.org/covid-19/pipeline/?avance-all&type - all & status - CE-IVD & section - immunoassays # diag_tab

Development of the CNR on the taking of samples and the sensitivity of RT-PCR tests for the detection of SARS-CoV 2 (05/09/2020)

Inventory of kits / reagents evaluations by CNR Lyon / IP respiratory viruses (version of 05/14/2020)

- Institut Pasteur reports on:
  - Thermofisher V1 kit
  - Thermofisher V2 kit
  - R-Biopharm kit (RIDA GENE)
  - NOVACYT kit (genesis)
  - Eurofins Biomnis kit
  - Amplidiag kit - Mobidiag
  - Genefirst kit - PrimaDiag
  - Bio-T SARS-CoV-2 kit - Biosellal
  - Bio-T Covid-19 kit - Biosellal
  - ILAMP Novel kit - Ionebio
  - Lyra® kit - Quidel corporation
  - IDNCOV-2 kit - idSOLUTIONS
  - OPTI kit - OPTIMedical
  - EXCILONE SARL kit
  - BIOMAXIMA kit
  - FosunPharma Diagnostics kit
  - IDVet kit
  - Novodiag kit - Mobidiag
  - Eurobio Scientific kit
  - Q-sens kit 2019 - Mediane France
  - Multiplex Digital kit - Stilla
  - SmartAmp kit - EMG / Mse
  - IDNCOV-2d kit (s) - Id Solutions
  - Tristar T-Kit Bio kit - Biosellal
  - RealAccurate Quadruplex kit - AirDiag_Pathofinder
- Lyon reports on:
  - Xpert® kit - Cepheid
  - CerTest kit - BD
  - BIOGX kit - Launch Diagnostics
  - ARGENE kit - bioMérieux
  - COBAS kit - Roche Diagnostics
  - VitaPCR ™ kit - BioSynex
  - RealStar®SARS-CoV-2 kit - AltonaDiagnostic
  - BD and R-Biopharm coupled kit
  - VIASURE kit - Orgentec
  - BioFire® kit - bioMérieux on FilmArray®Torch
  - Bosphore® kit - Launch Diagnostics
  - Combo2Screen kit - AB
  - kit: Simplexa ™ - Diasorin Molecular
  - Sansure Biotech kit

- Multiplex Digital kit - Stilla

Report available on:

https://www.sfm-microbiologie.org/2020/05/11/covid-19/

APPLICATION & TECHNICAL NOTES

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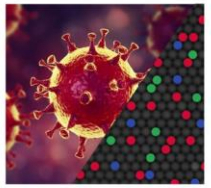
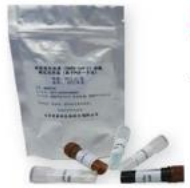
A 3-color
Crystal Digital PCR™ kit
for detection of COVID-19



Development of one-step RT-dPCR models for COVID-19 detection

The 2019-2020 outbreak of COVID-19 caused by the SARS-CoV-2 virus first reported in Wuhan, Hubei, China has been declared a pandemic by the World Health Organization. To facilitate the action of health authorities, the development of robust laboratory tests is of primary importance. Using the numerous publicly accessible SARS-CoV-2 and SARS-related sequences, several PCR-based assays specific for SARS-CoV-2 have been designed (Chan et al., 2020). The Naica compatible 3-color Crystal Digital PCR™ kit (Figure 1), developed by ApexBio (Hsinchu Science-based Industrial Park) includes primers and FAM- and HEX-labeled probes specific to two distinct regions (**ORF1ab** and Nucleocapside (N) genes, respectively) of the SARS-CoV-2 positive strand RNA genome. The 3rd channel of the Naica™ system has been used as an endogenous PCR reference detecting a human housekeeping gene with a Cy5-labeled probe. This single assay design permits the simultaneous detection of two independent SARS-CoV-2 sequences reported as conserved while concurrently monitoring PCR effectiveness using the third channel of detection (Figure 2).



apexbio

Kit components

- dPCR master mix1
- dPCR master mix2
- Primers and probes mix
- SARS-CoV-2 positive control
- SARS-CoV-2 negative control

Figure 1: The RUO ApexBio-developed ready-to-use kit contains all reagents required to perform a one-step RT 3-color Crystal Digital PCR™ on the Naica™ System.

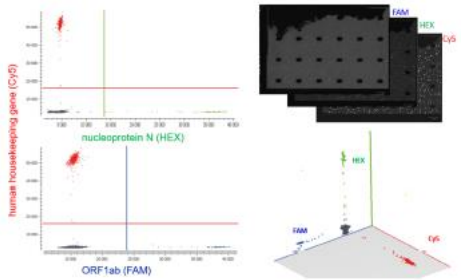


Figure 2: Crystal Miner-generated 2D (left) and 3D dot plots (right) and crystal droplet images obtained on positive controls containing human RNA and synthetic target sequences of the RUO RT-dPCR SARS-CoV-2 detection kit.

Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, Fung AY, Ng AC, Zou Z, Tsoi HW, Choi GK, Tam AR, Cheng VC, Chen KH, Tsang OT, Yuen KY. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens. J Clin Microbiol. 2020 Mar 4.

Sensitive and specific detection of COVID-19

An experimental model containing synthetic sequences targeted by the SARS-CoV-2 detection kit was serially diluted and seven dilution points were assessed in triplicate. A total of 1ng of human RNA was added to each replicate. The results indicated a robust and specific detection of SARS-CoV-2 sequences down to 0.6 copies per µl of positive control (5 copies per 25µl reaction) of the ORF1ab gene and down to 0.9 copies per µl of positive control (7 copies per 25µl reaction) of the Nucleocapside (N) gene in all tested samples. Further dilutions showed an extremely sensitive but stochastic detection **down** to 0.25 copies per µl of positive control (2 copies per 25µl reaction) for both genes (Figure 3). In parallel, a total of 15 controls containing only human RNA were tested as negative controls and no false positives were observed.

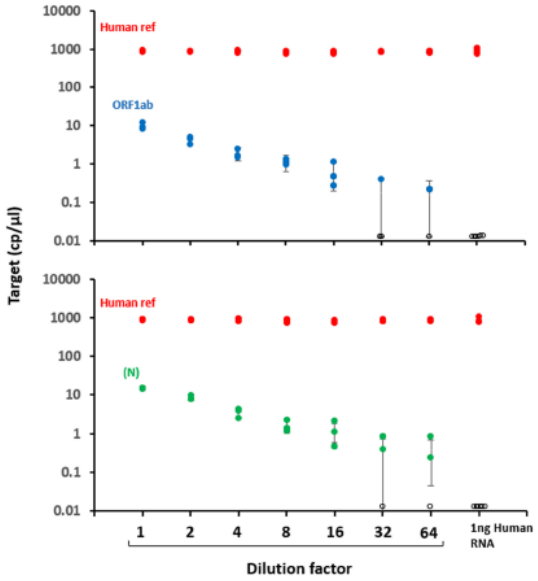


Figure 3: Sensitivity of the 3-color RUO RT Crystal Digital PCR™ kit for SARS-CoV-2 detection. Serial dilutions of SARS-CoV-2 synthetic targets were assayed in triplicate in a background of 1ng of human RNA. A total of 8µl of positive controls was added to each 25µl reaction. The vertical bars represent the theoretical 95% Poisson confidence intervals for the pool of 3 replicates. The empty circles represent replicates where SARS-CoV-2 sequences were not detected.

LA

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R assays
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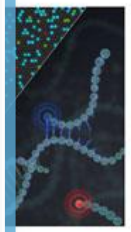

STILLA

Crystal Digital PCR VS Quantitative PCR

	Quantitative PCR	Digital PCR
Process	• Same Sample preparation methods and reagents • Similar initial sample volumes • Capability of multiplexing (amplifying several different DNA sequences simultaneously)	
Analysis	• **Standard curve required**	• **No standard curve required**
Results	• **Relative quantification** • **Reproducible results rely on human expertise**	• **Absolute quantification** • **Lower variability**
Usage	• Monitoring of real-time reaction efficiency • Relative gene expression if differences are >2-fold	• **High sensitivity and reproducibility** • **Rare** allele detection • **Easy** to use

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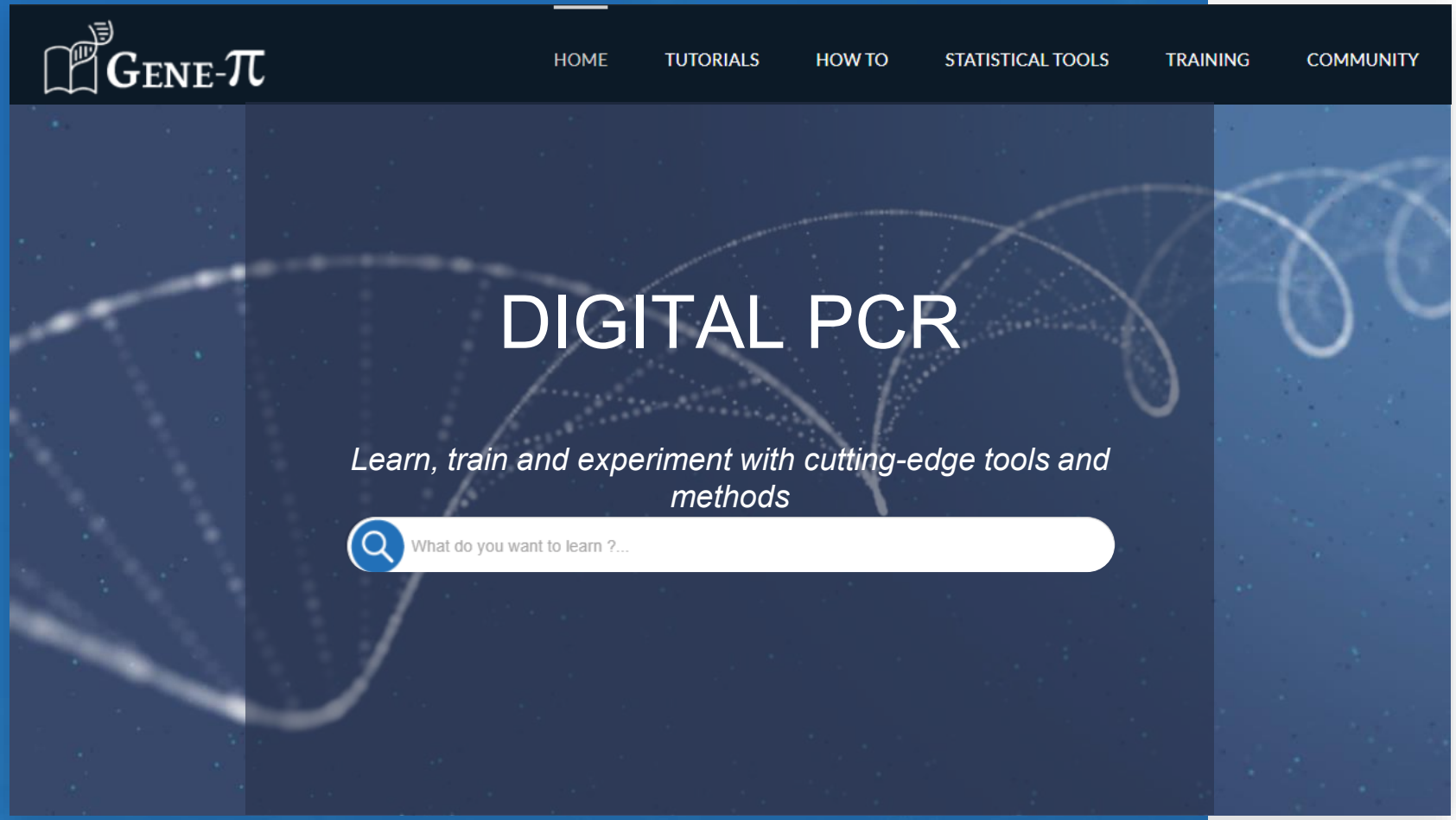

LAUNCH IN MARCH 2019:

- **3 tutorials**
  - Rare Mutation Detection
  - CNV
  - Drop-off Assay
- **1 video**
- **14 how to's**
- **3 memos**
- **3 online statistical tools**
  - Poisson Law
  - CNV
  - Limit of Blank/Limit of Detection