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

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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

  Winichakoon P, et.al. Journal of Clinical Microbiology Feb 2020

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

  Arima Y, Emerg Infect Dis. 2020
  Xie X, et.al. Radiology. 2020

Necessity to develop more sensitive detection tools

NEXT GENERATION OF PCR
Digital PCR

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

OUR MISSION:
MAKE DIGITAL PCR A LAB COMMODITY
NEXT GENERATION OF PCR
Digital PCR

• Digital PCR could be a valuable asset in the COVID-19 battle
**PRINCIPLE OF DIGITAL PCR**

**PARTITIONING**

Wild-type DNA

**PCR**

**READING & ANALYSIS**

RESULTS

2636 cp/µL with 2.2 % uncertainty

**POISSON STATISTICS**

\[
\frac{N_{\text{pos}}}{N_{\text{tot}}}
\]
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™

Step 2.1 – Partition
• 1-3 chips and 1-12 samples / run
• Contactless fluid injection

Step 2.2 – Amplify

Sapphire Chip (consumable)
Naica™ Geode
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
- 15 s
- 15 s
- 5 min

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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 mix 1
- dPCR master mix 2
- 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

- Chest CT abnormalities
- Viral RNA extraction
- Nasopharyngeal swab
- qPCR negative in first instance

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

Chest CT abnormalities

Viral RNA extraction: qPCR negative in first instance

Nasopharyngeal swab

Naica System

COVID-19 Detection kit

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

qPCR negative in first instance

Viral RNA extraction

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Viral charge in Crystal Digital PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>negative</td>
</tr>
<tr>
<td>Patient 2</td>
<td>positive</td>
</tr>
<tr>
<td>Patient 3</td>
<td>positive</td>
</tr>
<tr>
<td>Patient 4</td>
<td>negative</td>
</tr>
<tr>
<td>Patient 5</td>
<td>negative</td>
</tr>
<tr>
<td>Patient 6</td>
<td>negative</td>
</tr>
<tr>
<td>Patient 7</td>
<td>negative</td>
</tr>
<tr>
<td>Patient 8</td>
<td>positive</td>
</tr>
<tr>
<td>Patient 9</td>
<td>positive</td>
</tr>
<tr>
<td>Patient 10</td>
<td>negative</td>
</tr>
</tbody>
</table>

4 patients on 10 were retrieved positives

Could bring relevant clinical information at the first onset of the infection
### COVID-19 quantification by cdPCR

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

<table>
<thead>
<tr>
<th>qPCR</th>
<th>Number of patient tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (Undetermined)</td>
<td>15</td>
</tr>
<tr>
<td>Positive (Ct&lt;35)</td>
<td>12</td>
</tr>
<tr>
<td>Doubtful (Ct&gt;34)</td>
<td>18</td>
</tr>
</tbody>
</table>

- One recall, determined positive by cdPCR

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

**Nucleoprotein N**

\[ y = 6 \times 10^8 e^{-0.619x} \]

\[ R^2 = 0.9166 \]

**ORF1ab**

\[ y = 4 \times 10^8 e^{-0.616x} \]

\[ R^2 = 0.8854 \]

Full correlation cannot be reached, 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 MEPI, IHU Méditerranée Infection, Marseille.
COVID-19 quantification by cdPCR in high Ct qPCR data

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<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Numéro</th>
<th>CT qPCR</th>
<th>Viral charge determined in cdPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.32</td>
<td>neg</td>
</tr>
<tr>
<td>2</td>
<td>35.53</td>
<td>pos</td>
</tr>
<tr>
<td>3</td>
<td>35.15</td>
<td>pos</td>
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<tr>
<td>4</td>
<td>34.65</td>
<td>neg</td>
</tr>
<tr>
<td>5</td>
<td>36.92</td>
<td>neg</td>
</tr>
<tr>
<td>6</td>
<td>36.56</td>
<td>neg</td>
</tr>
<tr>
<td>7</td>
<td>36.75</td>
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<tr>
<td>8</td>
<td>35.17</td>
<td>Pos</td>
</tr>
<tr>
<td>9</td>
<td>34.37</td>
<td>pos</td>
</tr>
<tr>
<td>10</td>
<td>37.43</td>
<td>pos</td>
</tr>
<tr>
<td>11</td>
<td>34.95</td>
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<td>12</td>
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<tr>
<td>15</td>
<td>36.66</td>
<td>neg</td>
</tr>
<tr>
<td>16</td>
<td>34.3</td>
<td>pos</td>
</tr>
<tr>
<td>17</td>
<td>36.96</td>
<td>neg</td>
</tr>
<tr>
<td>18</td>
<td>36.47</td>
<td>pos</td>
</tr>
</tbody>
</table>

*IC highlight a possible Collection/Extraction issue

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

Crystal Digital PCR Covid-19 detection kit is an easy and fast solution to investigate difficult to interpret high Ct value qPCR data.
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 easy and fast solution to investigate difficult to interpret high Ct value qPCR data.
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://solidarite-sante.gouv.fr/IMG/pdf/laet_reactifs_diagnostic-rt-pcr.pdf

List of CE marked COVID-19 reagents available worldwide: pdf format to download dated 03/05/2020 here or on https://www.fhmdh.org/covid-19/pipelines?avance=av1&type=all&status=CE-VID&section=immunodiagnostic&dialog=true

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

Inventory of lists / reagents evaluations by CNR Lyon / P/ respiratory virus (version of 09/14/2020)

- Institut Pasteur reports on:
  - ThermoFisher V2 kit
  - ThermoFisher V2 kit
  - R-Biopharm kit (DBA GENE)
  - Nuclease It (generall)
  - Eurofines Bioinnov kit
  - AmpliTag kit - Mobidiag
  - GeneFire1 kit - Primatag
  - Bio-T SARS-CoV-2 kit - Bioselal
  - Bio-T Covid-19 kit - Bioselal
  - ILAMP Novel kit - Innbio
  - LyraF kit - Guideli corporation
  - IDOMIC2 kit - RIVRISOLVATIONS
  - CRIS-kit - CRISPTMEDICAL
  - EPILOKANE SARI kit
  - MIDMAXIMA kit
  - FocusPharma Diagnostics kit
  - Oiet kit
  - Novodiag kit - Mobidiag
  - Eurobio Scientific kit
  - Multiplex Digital kit - Stilla

- Lyon reports on:
  - Upert kit - Cepheid
  - Gerta kit - TIB
  - BOOST kit - Launch Diagnostics
  - AMYDRA kit - InnoMerieux
  - COBAS kit - Roche Diagnosis
  - VitaPCR kit - BioSyme
  - Raaat/RaatiSARS-CoV-2 kit - ABBOTT Diagnostics
  - 96 and 9 Biornform couvkit
  - VasaRED kit - Ortho
  - BioFire kit kit - BioMerieux on Filmarray® torch
  - Biepharmkit - launch diagnostics
  - CombiDiagnostics kit
  - Air Sampling kit
  - Spectr-Molecular
  - Specimen Processing kit

Report available on:
https://www.sfm-microbiologie.org/2020/05/11/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-Cov-1 datasets, several PCR-based assays specific for SARS-Cov-2 have been designed (Yun et al., 2020). The common primer probe oligo collection (Figure 1), developed by AlexBio (Hsinchu Science-based Industrial Park), includes primers and FAM and HEX-labeled probes specific to two distinct regions (ORF1ab and Nucleocapsid (N) gene), respectively, of the SARS-Cov-2 positive strand RNA genome. The 3rd channel of the TaqMan™ system is used as an endogenous PCR control 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 continuously monitoring PCR effectiveness using the third channel of detection (Figure 2).

Sensitive and specific detection of COVID-19

An experimental setup containing synthetic sequences targeted by the SARS-Cov-2 detection kit was serially diluted and seven dilution steps were assessed in triplicate. A total of 10 ng of human RNA was added to each replicate. The results indicated a robust and specific detection of SARS-Cov-2 sequences down to 50 copies per 10 μl of positive control (5 copies per 25 μl reaction) of the ORF1ab gene and down to 0.5 copies per 25 μl reaction of the Nucleocapsid (N) gene in all tested samples. Further dilutions showed an extremely sensitive but stochastic detection down to 0.05 copies per 10 μl of positive control (2 copies per 25 μl reaction) for both genes (Figure 3). In parallel, a total of 10 controls containing only human RNA were tested as negative controls and no false positives were observed.
## Crystal Digital PCR VS Quantitative PCR

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

LEARNING CENTER:
www.gene-pi.com