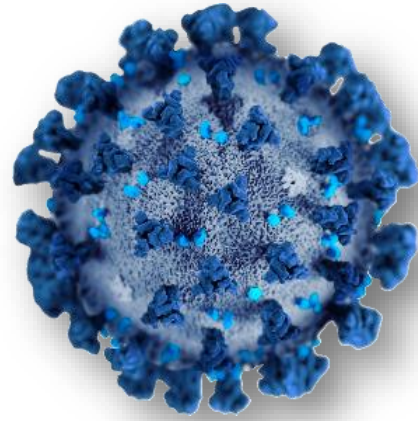




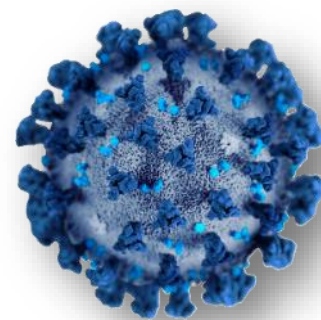
Pivoting from Cancer to COVID-19 in a global pandemic

Paul Hofman, MD, PhD

Disclaimer

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Speaker was provided an honorarium by Thermo Fisher Scientific for this presentation.

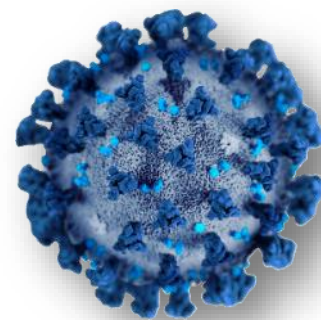


The COVID-19 crisis in Nice, France, and the impact in a pathology lab

SARS-CoV-2 diagnosis set-up in a pathology lab: experience of the LPCE

Current development using the NGS approach for SARS-CoV-2 assessment

Perspective



The COVID-19 crisis in Nice, France, and the impact in a pathology lab

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Global Cases
144 800 467

Cases by Country/Region
/Sovereignty

31 929 601 US

16 263 695 India

14 167 973 Brazil

5 469 674 France

4 682 573 Russia

4 501 382 Turkey

4 413 834 United Kingdom

3 920 945 Italy

3 456 886 Spain

3 254 609 Germany

2 796 768 Argentina

2 731 256 Poland

2 720 619 Colombia

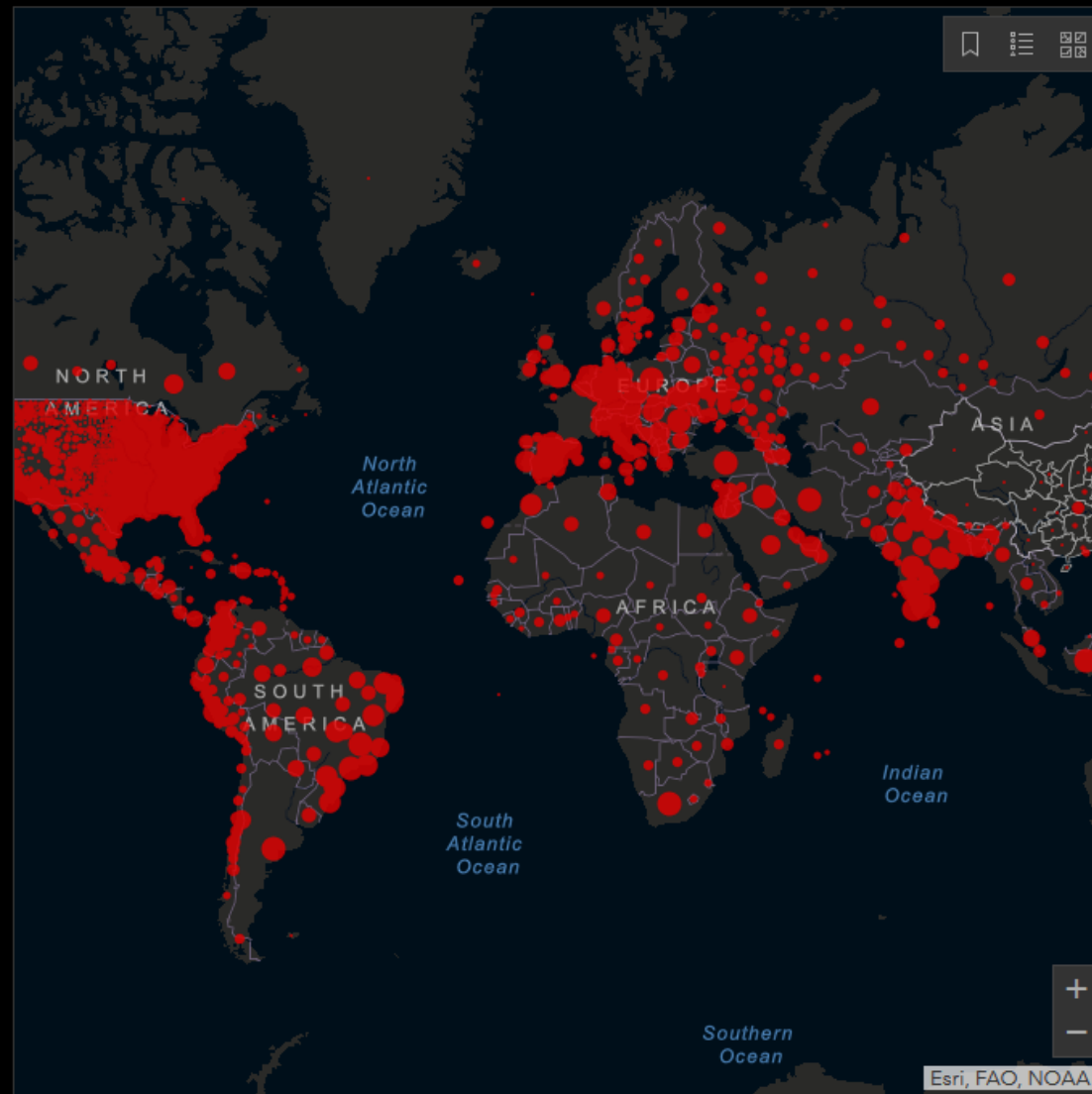
2 335 905 Iran

2 319 519 Mexico

2 058 301 Ukraine

Admin0

Last Updated at (M/D/YYYY)
4/23/2021 10:20 AM



Global Deaths
3 072 614

570 345 deaths
US

383 502 deaths
Brazil

214 095 deaths
Mexico

186 920 deaths
India

127 597 deaths
United Kingdom

118 357 deaths
Italy

105 328 deaths
Russia

102 323 deaths
France

Global Deaths

Total Test Results in US
421 219 495

58 202 413 tests
California US

49 426 695 tests
New York US

22 537 890 tests
Texas US

22 120 098 tests
Florida US

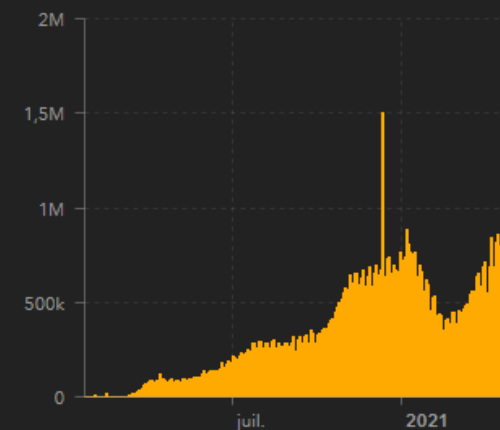
21 920 359 tests
Illinois US

20 599 779 tests
Massachusetts US

13 217 769 tests
New Jersey US

12 617 144 tests
Pennsylvania US

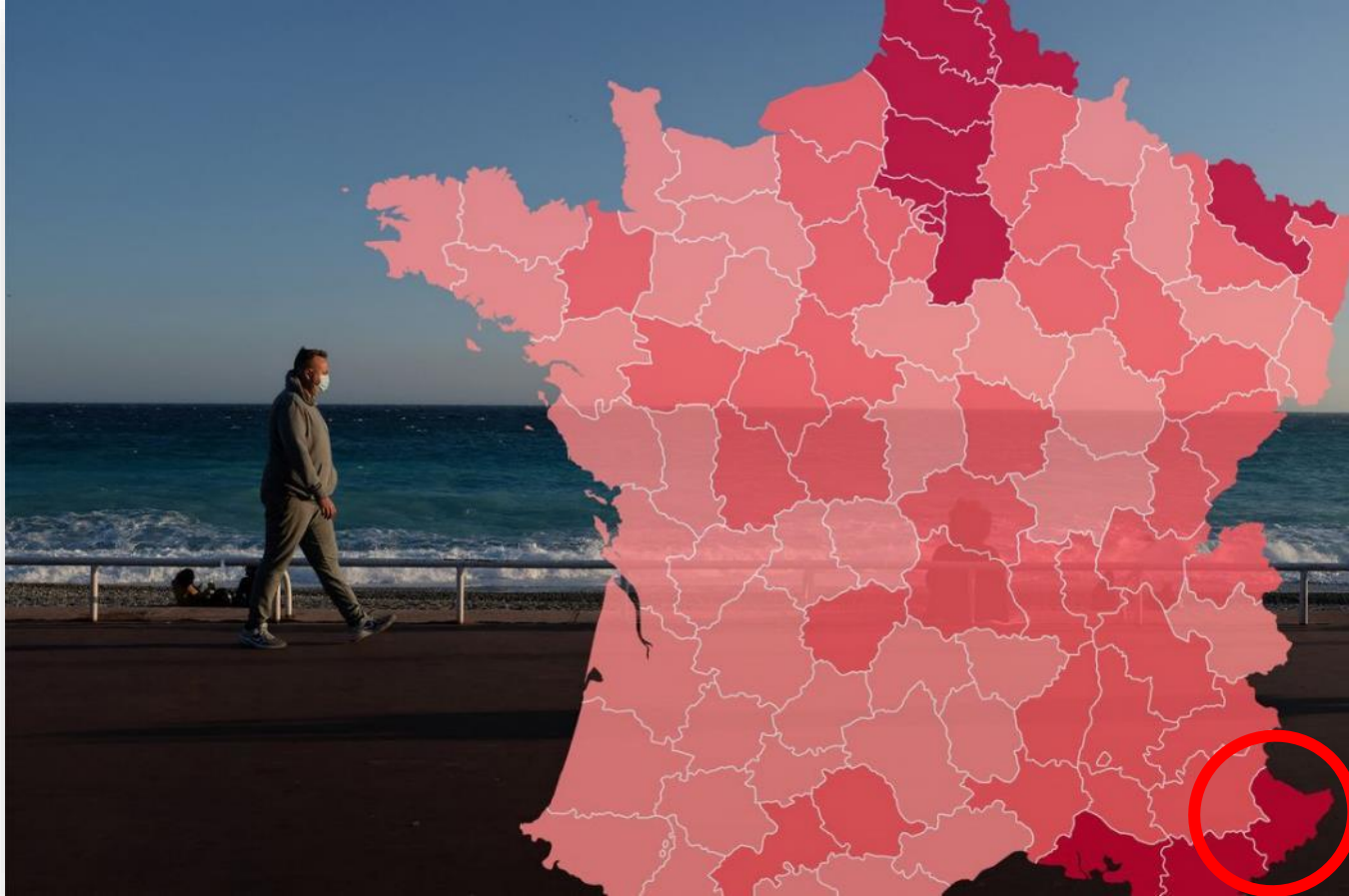
US Test Res...

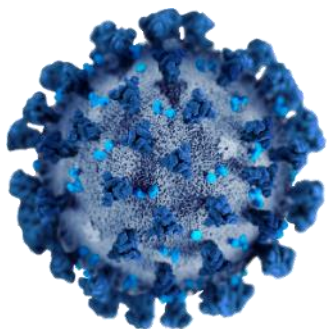


Daily Cases

Walk of the SARS CoV 2 on the « promenade des Anglais »

Higher incidence of the virus in France (March 2021)





Nice city



Louis Pasteur Hospital, University of Nice, France

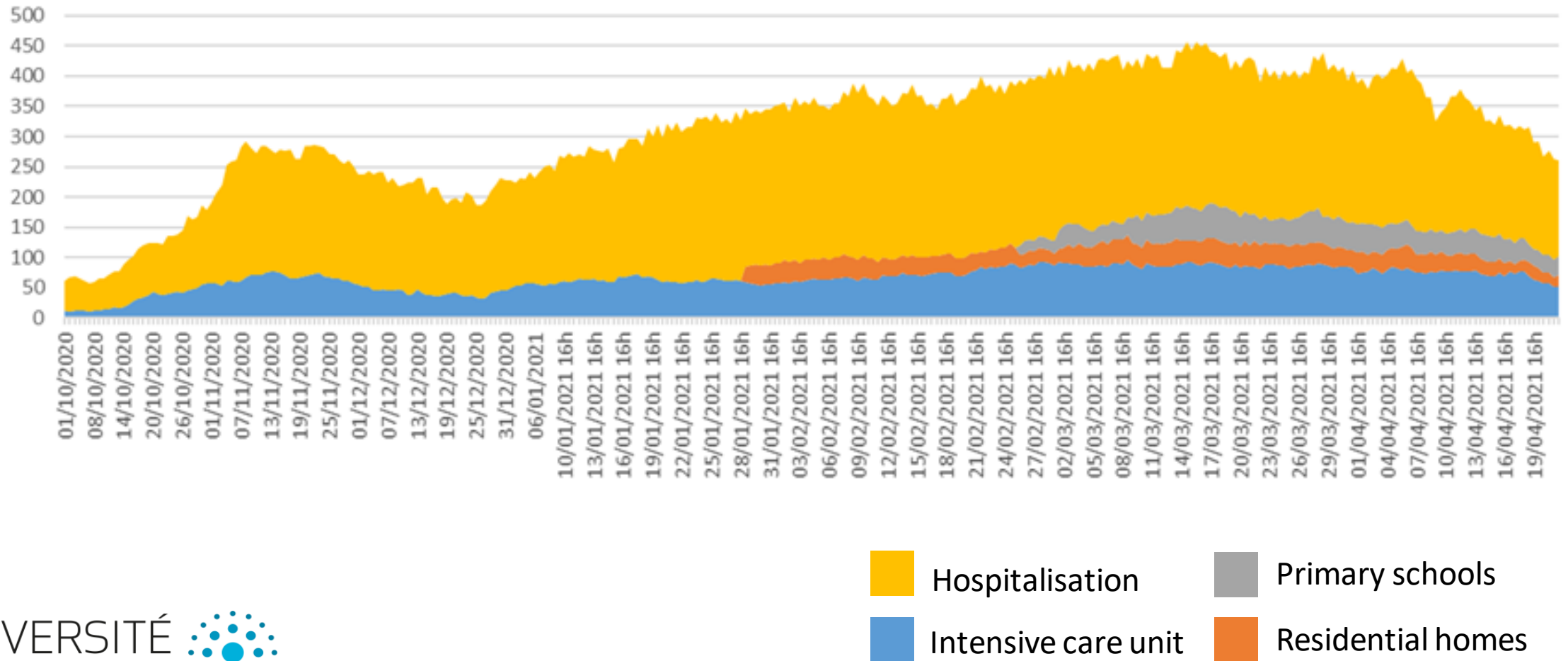


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Evolution of COVID-19 cases in Nice, France

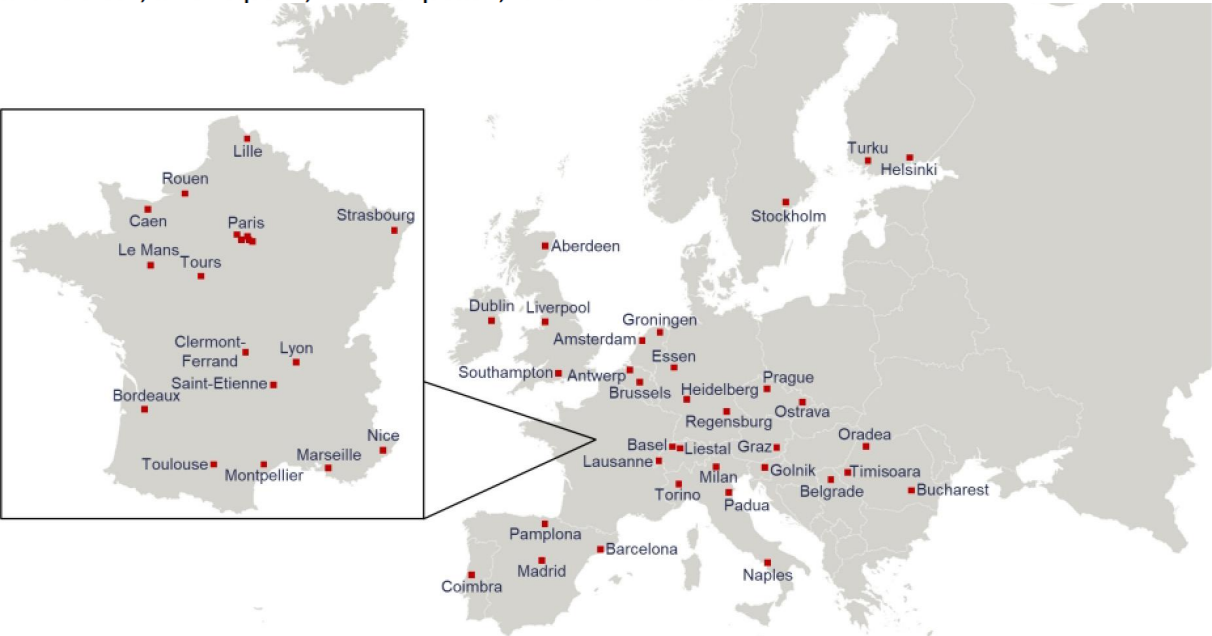
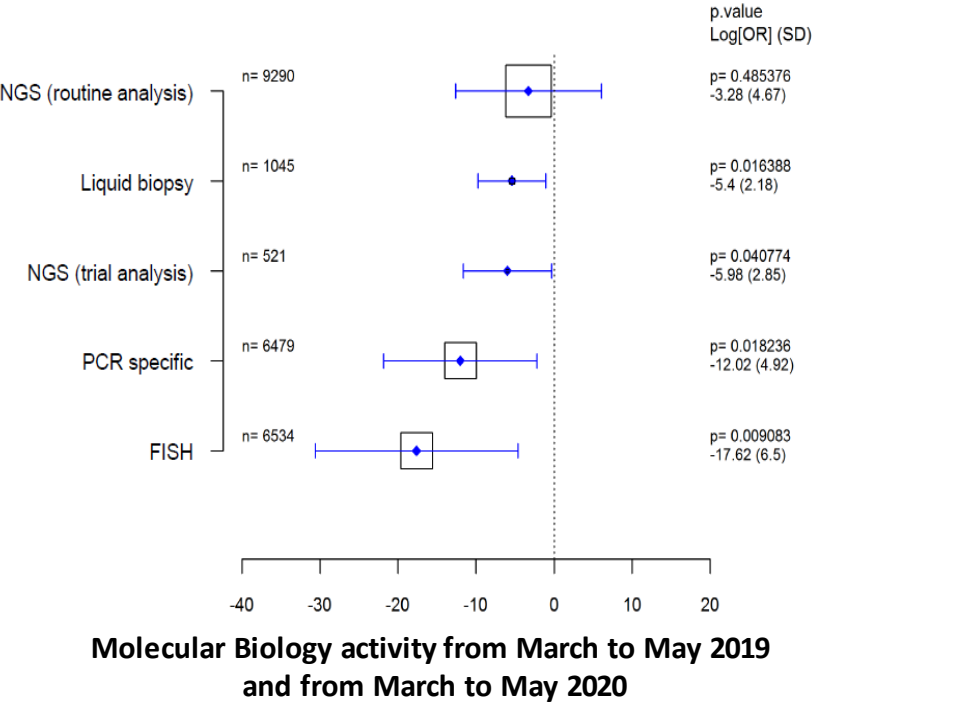
October 1, 2020 to April 19, 2021



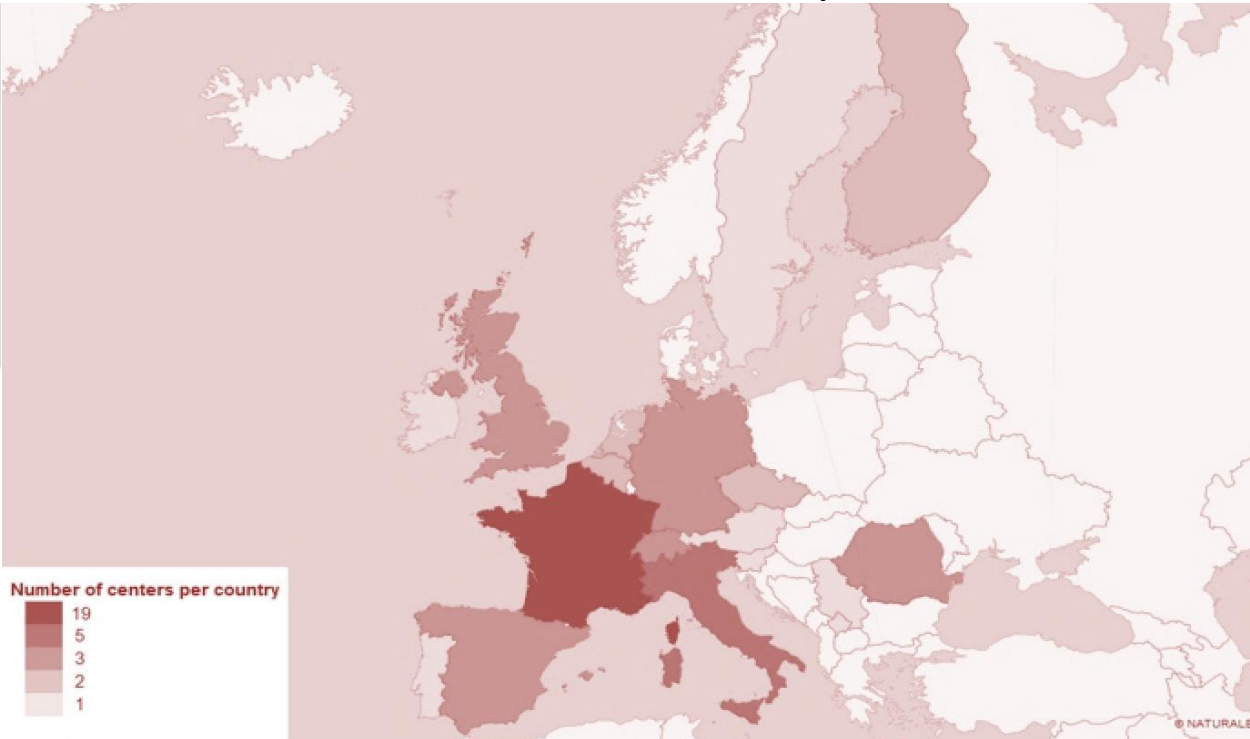
ORIGINAL RESEARCH

Clinical and molecular practice of European thoracic pathology laboratories during the COVID-19 pandemic. The past and the near future

P. Hofman^{1†*}, M. Ilie^{1†}, E. Chamorey^{2†}, P. Brest^{3†}, R. Schiappa², V. Nakache¹, M. Antoine⁴, M. Barberis⁵, H. Begueret⁶, F. Bibeau⁷, C. Bonnetaud¹, P. Boström⁸, P. Brousset⁹, L. Bubendorf¹⁰, L. Carvalho¹¹, G. Cathomas¹², A. Cazes¹³, L. Chalabreysse¹⁴, M.-P. Chenard¹⁵, M.-C. Copin¹⁶, J.-F. Côté¹⁷, D. Damotte¹⁸, L. de Leval¹⁹, P. Delongova²⁰, V. Thomas de Montpreville²¹, A. de Muret²², A. Dema²³, W. Dietmaier²⁴, M. Evert²⁴, A. Fabre²⁵, F. Forest²⁶, A. Foulet²⁷, S. Garcia²⁸, M. Garcia-Martos²⁹, L. Gibault³⁰, G. Gorkiewicz³¹, D. Jonigk³², J. Gosney³³, A. Hofman¹, I. Kern³⁴, K. Kerr³⁵, M. Kossai³⁶, M. Kriegsmann³⁷, S. Lassalle¹, E. Long-Mira¹, A. Lupo¹⁸, A. Mamilos²⁴, R. Matěj³⁸, J. Meilleroux⁹, C. Ortiz-Villalón³⁹, L. Panico⁴⁰, A. Panizo⁴¹, M. Papotti⁴², P. Pauwels⁴³, G. Pelosi⁴⁴, F. Penault-Llorca³⁶, O. Pop⁴⁵, N. Poté¹³, S. R. Y. Cajal⁴⁶, J.-C. Sabourin⁴⁷, I. Salmon⁴⁸, M. Sajin⁴⁹, S. Savic-Prince¹⁰, H.-U. Schildhaus⁵⁰, P. Schirmacher³⁷, I. Serre⁵¹, E. Shaw⁵², D. Sizaret²², A. Stenzinger³⁷, J. Stojic⁵³, E. Thunnissen⁵⁴, W. Timens⁵⁵, G. Troncone⁵⁶, C. Werlein³², H. Wolff⁵⁷, J.-P. Berthet⁵⁸, J. Benzaquen⁵⁹, C.-H. Marquette⁵⁹, V. Hofman^{1†} & F. Calabrese^{60†}

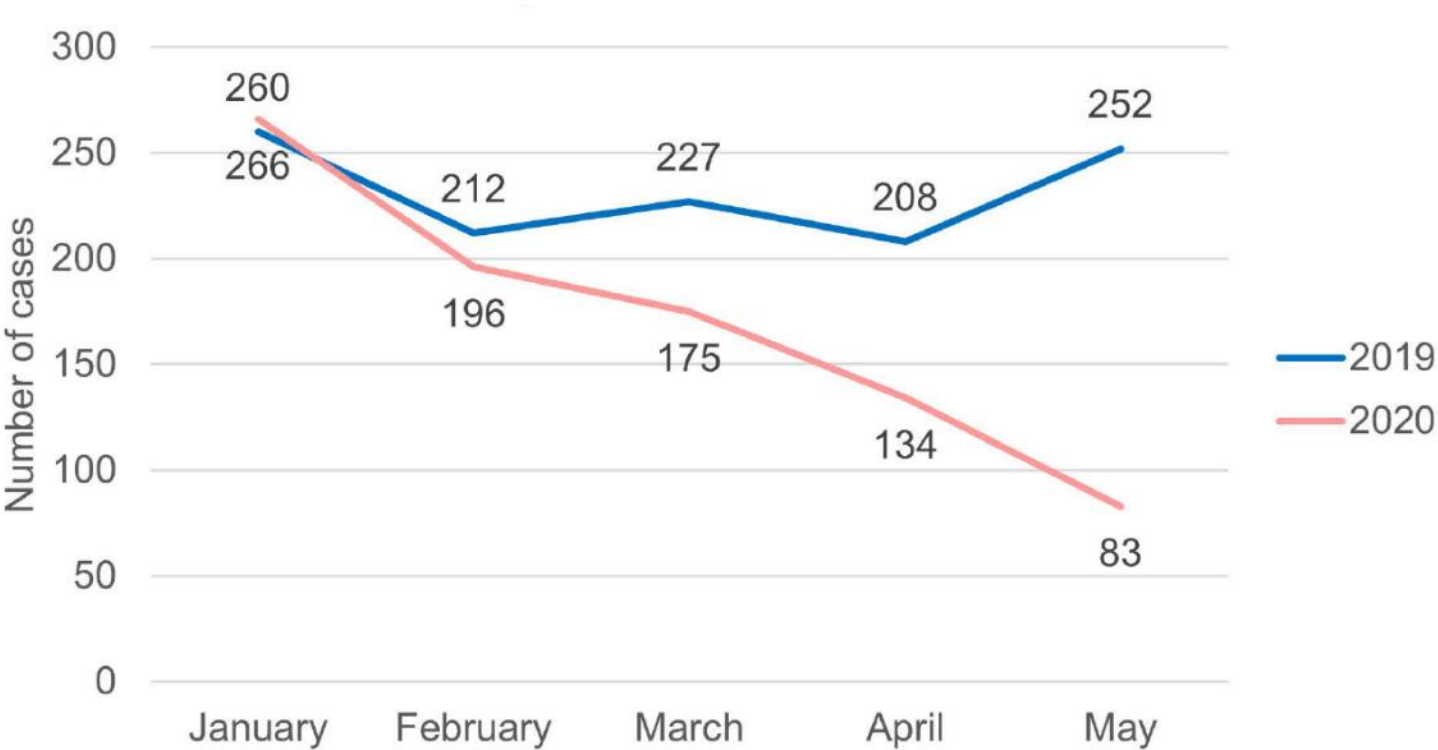


Pathology laboratories participating to the survey

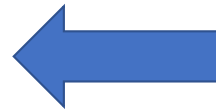


Activity of thoracic cancer surgeries in the South Region, France

Between 2019 and 2020



Nice (LPCE)
molecular pathology platforms



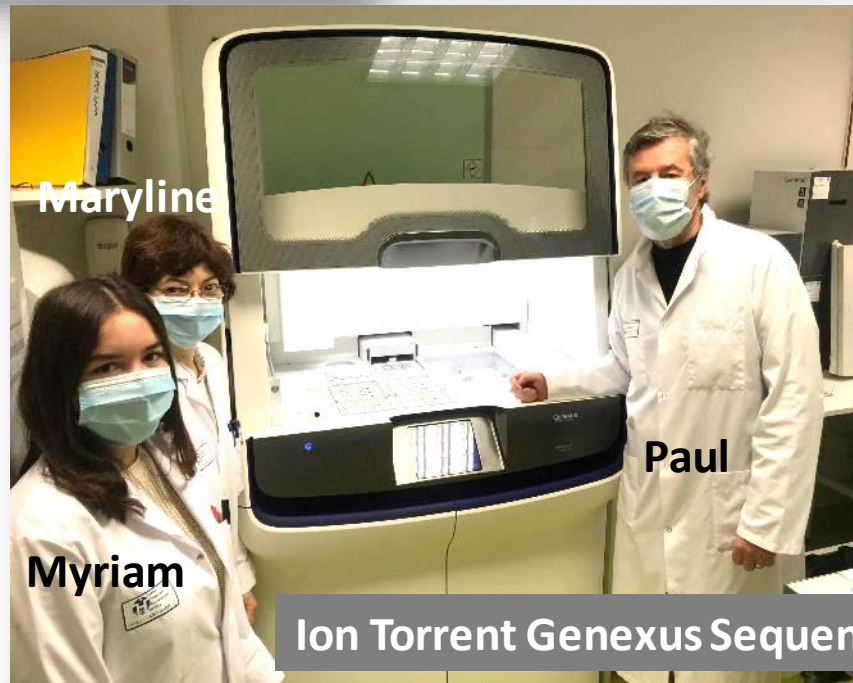
Virginie

Chef & GeneStudio NGS



Olivier

Targeted sequencing
Idylla (Biocartis)



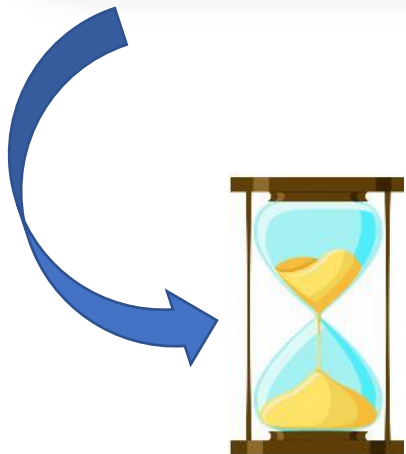
Maryline

Paul

Myriam

Ion Torrent Genexus Sequencer

Targeted sequencing
Cobas (Roche)



Specimen to report in a single day with only two user touchpoints

Ion Torrent Genexus System

- Lysate from FFPE tissue
- Plasma
- Whole blood
- Peripheral blood lymphocyte (PBL)
- Lysate from fresh-frozen tissue
- Lysate from bone marrow

Nucleic acid purification and quantitation*

Ion Torrent™ Genexus™
Purification System (Available 2021)



2 hour turnaround time
12 FFPE (DNA and RNA)
6 Plasma

Genexus Software

Library preparation to variant interpretation

Report*

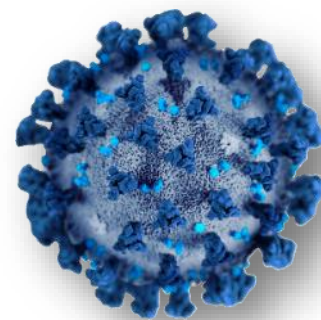
Ion Torrent™ Genexus™
Integrated Sequencer (November 2019)

**Ion Torrent™
GX5™ Chip:**
12–15M
reads/lane



14 hours for a single-lane run
(approx. 24 to 30 hours for full chip)

Up to 32 Samples per run



The COVID-19 crisis in Nice (France) and its impact in a pathology lab

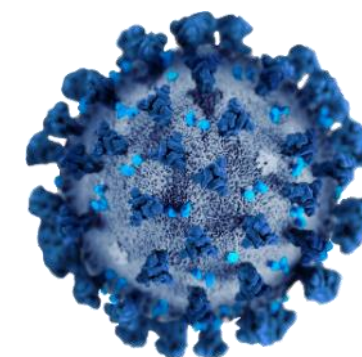
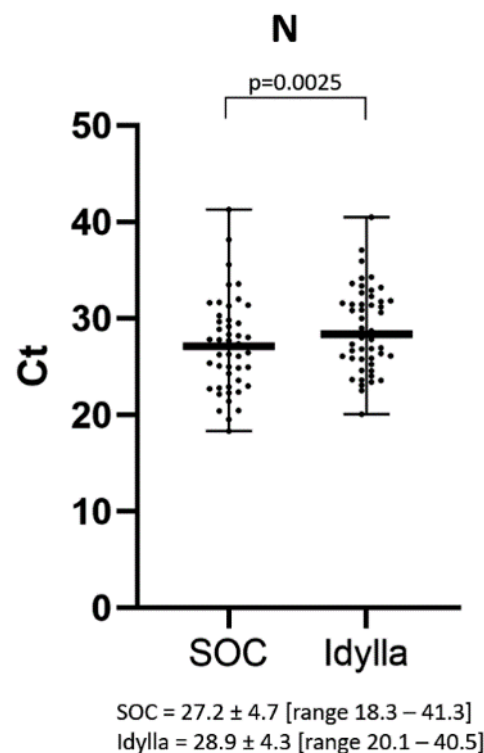
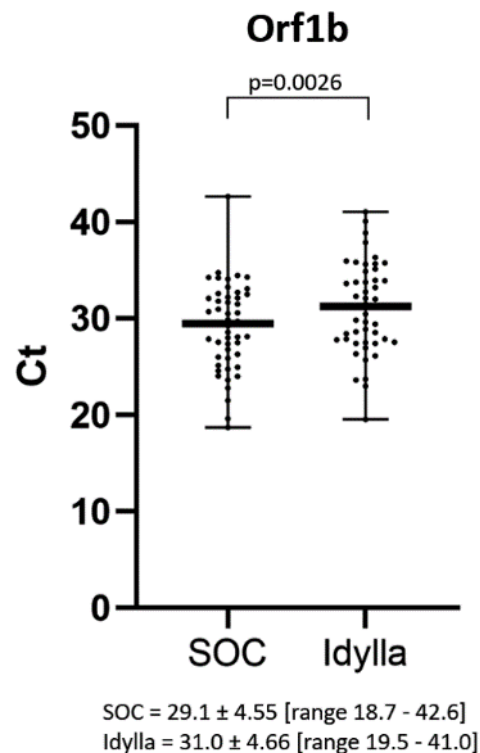
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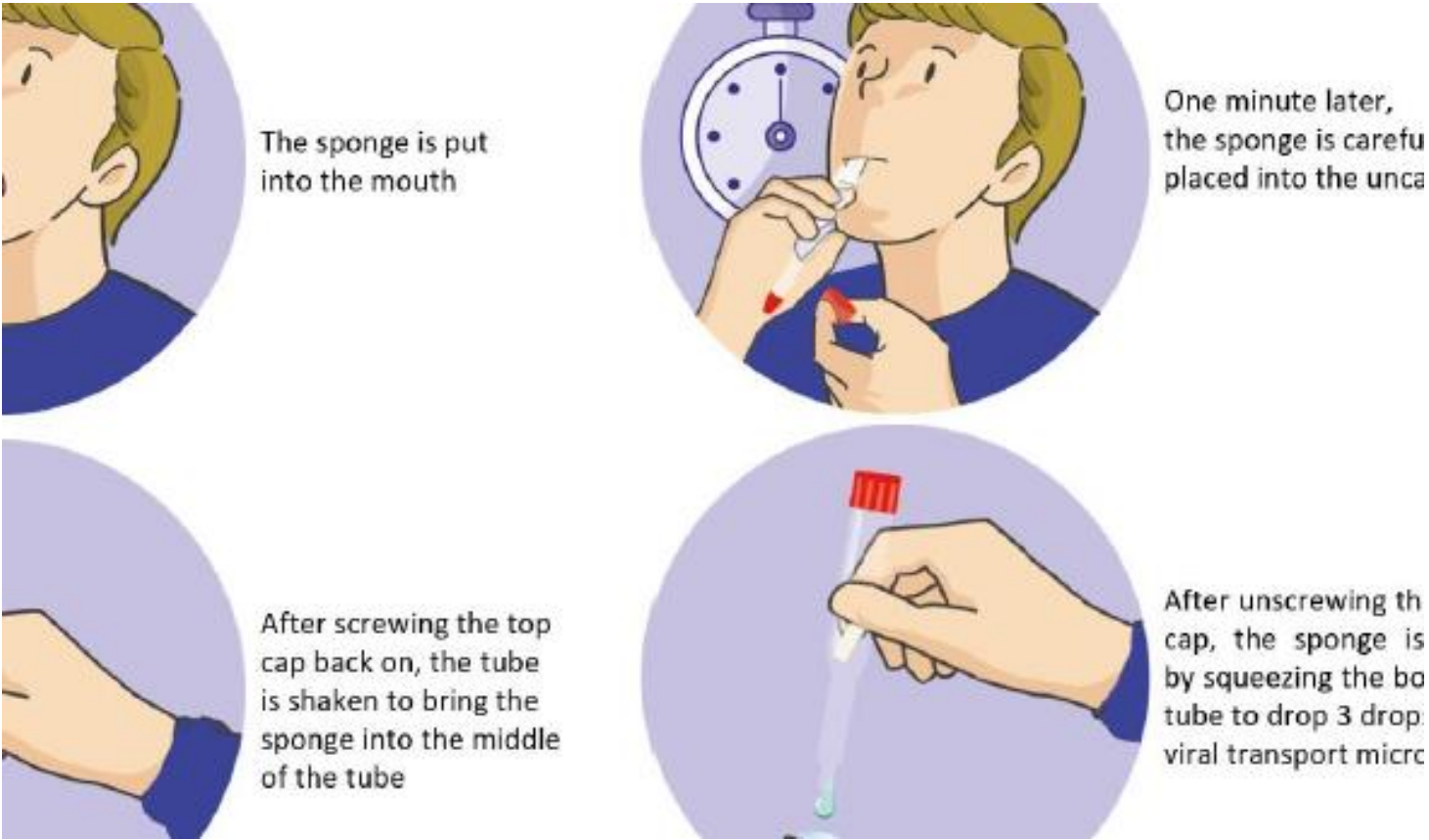
Setting up a collection of SARS-CoV-2 positive NSP samples and storage in an accredited biobank (BB-0033-00025)

First period

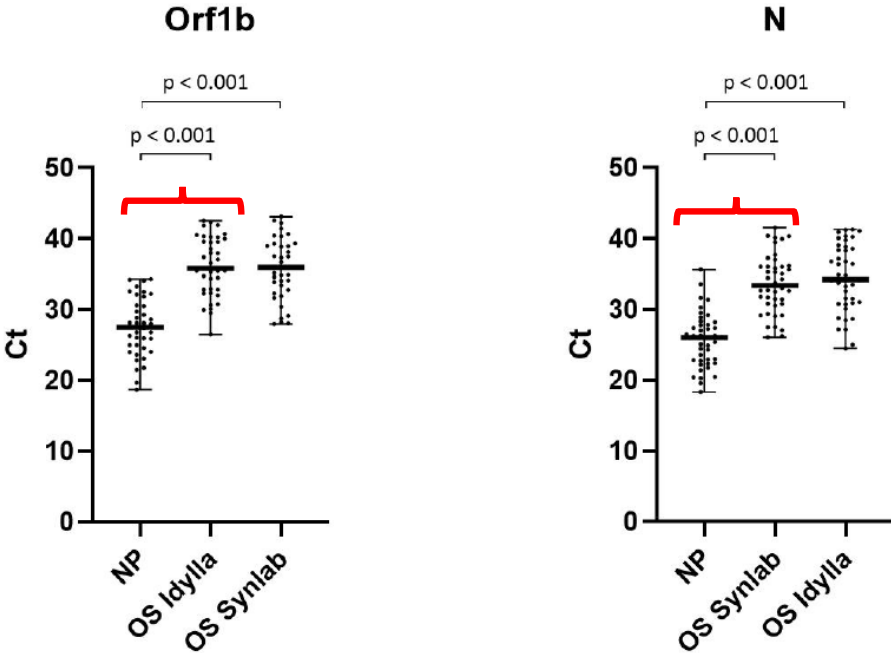


Setting up a collection of SARS-CoV-2 positive oral samples and storage in an accredited biobank (BB-0033-00025)

Second period



All-in-on saliva sampling technique with a sponge.
Usefulness of a double-capped plastic



Viral load of NasoPharyngeal and oral samples assessed by the Ct for the *Orf1b* and *N* genes

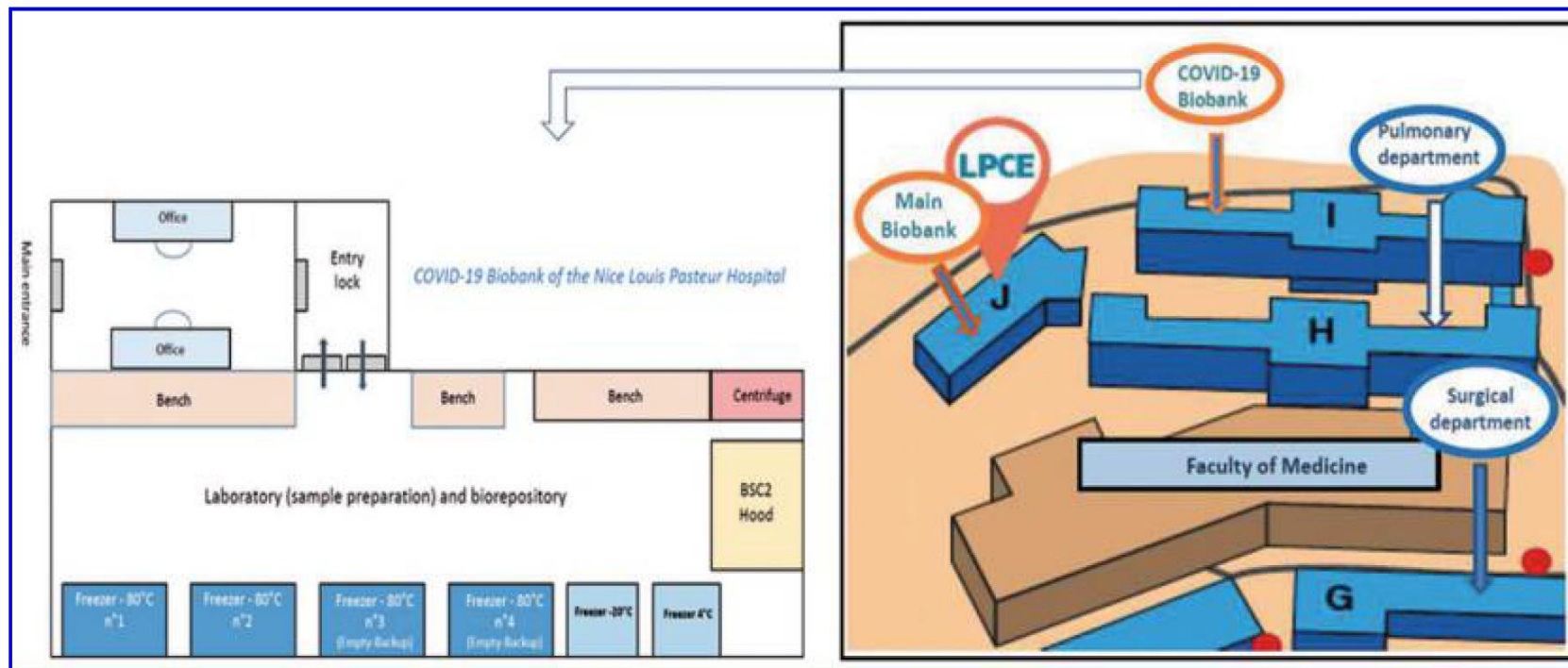
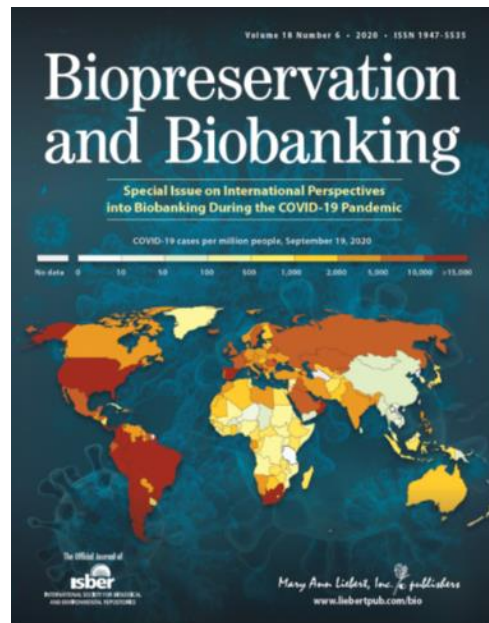


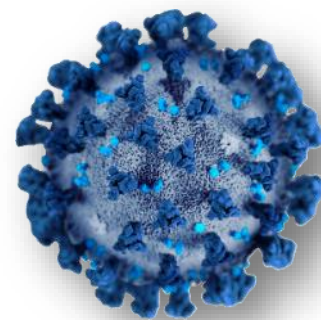
FIG. 1. Facilities of the COVID-19 Nice Louis Pasteur Hospital Biobank. LPCE, Laboratory of Clinical and Experimental Pathology.



BIOPRESERVATION AND BIOBANKING
Volume 00, Number 00, 2020
© Mary Ann Liebert, Inc.
DOI: 10.1089/bio.2020.0055

Establishment of a Collection of Blood-Derived Products from COVID-19 Patients for Translational Research: Experience of the LPCE Biobank (Nice, France)

Virginie Tanga,^{1,2} Sylvie Leroy,³ Julien Fayada,¹ Maram Hamila,¹ Maryline Allegra,¹ Zeineb Messaoudi,¹ Christelle Bonnetaud,¹ Virgine Lespinet,¹ Olivier Bordone,¹ Kevin Washetine,¹ Jennifer Griffonnet,³ Charlotte Maniel,³ Lorène Philibert,³ Eric Selva,¹ Jonathan Benzaquen,³ Marius Ilie,^{1,2} Elodie Long,^{1,2} Sandra Lassalle,^{1,2} Elisabeth Lantéri,^{1,2} Charles-Hugo Marquette,^{2,3} Véronique Hofman,^{1,2} and Paul Hofman^{1,2}



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London

Cite this as: *BMJ* 2020;371:m4857

<http://dx.doi.org/10.1136/bmj.m4857>

Published: 16 December 2020

NEWS BRIEFING

Covid-19: New coronavirus variant is identified in UK

England's health secretary, Matt Hancock, has told parliament that a new variant of covid-19 has been identified and may be driving infections in the south east, leading to headlines about "mutant covid." **Jacqui Wise** answers some common questions

Jacqui Wise



The 501.V2 and B.1.1.7 variants of coronavirus disease 2019 (COVID-19): A new time-bomb in the making?

Taha Bin Arif MBBS 

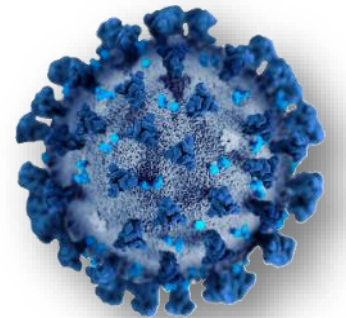
Department of Medicine, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan



Could it be that the B.1.1.7 lineage is more deadly?

Chia Siang Kow¹ and Syed Shahzad Hasan^{2,3}

¹School of Postgraduate Studies, International Medical University, Kuala Lumpur, Malaysia, ²School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom and ³School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, Australia



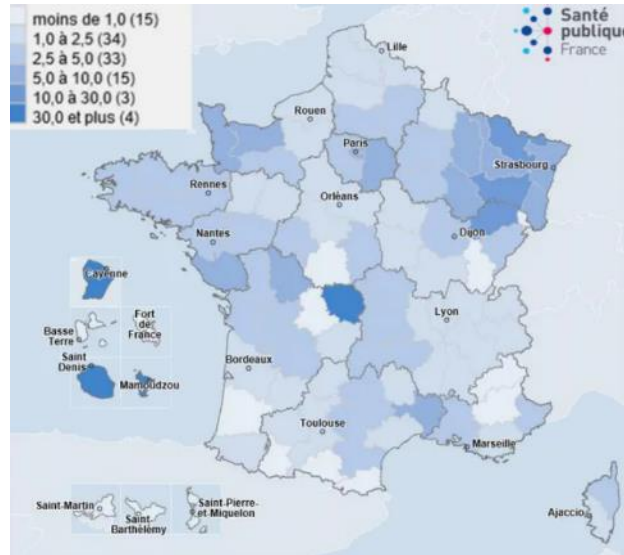
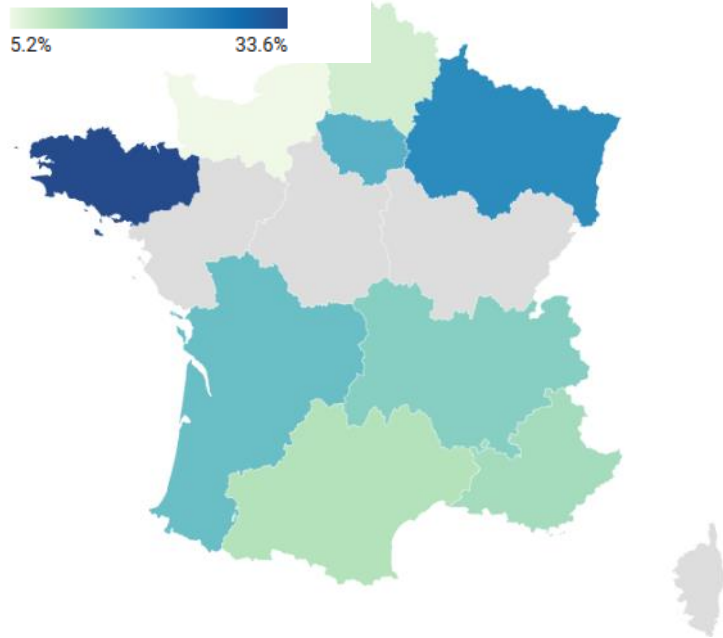
SARS COV 2 variants of concerns present in France

- B.1.17 (UK): **82% of variants in France** April 21, 2021
- 20H/501Y.V2 (South of Africa) and 20J/501Y.V3 (Brazil): **4.2 % of variants in France** April 21, 2021
- B.1.616 (ou 20C/655Y) (Britain, France): Not detected using SARS-CoV-2 RT-PCR from NSP swabs

Covid variants spread

Percentage of Covid tests that were UK, SA or BR variants

5.2% 33.6%



April 21, 2021, % of 20H/501Y.V2 and 20J/501Y.V3 variants among SARS-CoV-2 positive tests

B.1.617 (India)

April 29, 2021

Covid-19 dans le monde : le variant indien détecté en Belgique, chez des étudiants passés par Roissy

Vingt étudiants indiens ont été testés positifs à ce mutant du SARS-CoV-2. Ils ont été placés en quarantaine dans les villes flamandes d'Alost et de Louvain, où ils étaient arrivés mi-avril.

Le Monde

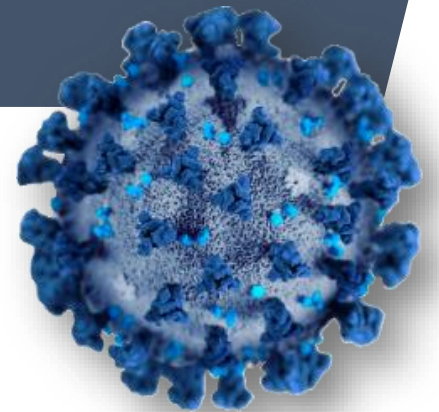


Key Questions around the new Coronavirus Strains

- How can we detect new strains?
- What is the origin of the new strain and how prevalent is it?
- Will current molecular or antigen-based tests miss detecting this strain?
- Does this strain spread more quickly?
- **Does this strain lead to higher disease severity?**
- **What is the vaccine efficacy against the new strain?**

**There are still
currently many
unknowns.**

Ongoing research is
needed to better
understand virus strain
evolution and spread.



Key questions on the new Coronavirus strains

- How can we detect
- What is the origin
prevalent is it?
- Will current methods
detecting this
- Does this strain
- **Does this strain**
- **What is the vaccine efficacy**

There are still
currently many
unknowns.

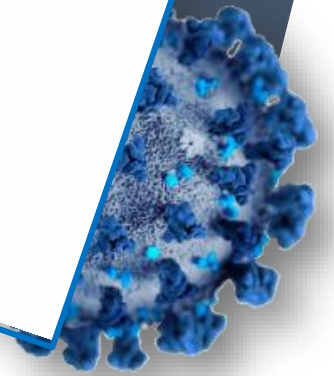
Research is

The NEW ENGLAND JOURNAL of MEDICINE
N Engl J Med. 2021 May 5. doi: 10.1056/NEJMc2104974

CORRESPONDENCE

Effectiveness of the BNT162b2 Covid-19 Vaccine
against the B.1.1.7 and B.1.351 Variants

*But what about the Brazilian
and Indian variants...?*





Retrospective analysis

Starting March 2021

From RT-PCR to NGS
assessment of SARS-CoV-2

*145 nasopharyngeal swabs samples
and 145 matched saliva samples
positives for RT-PCR SARS-CoV-2*

*Individuals tested from April 2020 to
January 2021*

*Samples collected and stored
at -80°C in the Biobank 0033-00025
University Côte d'Azur
(www.biobank-cotedazur.fr)*

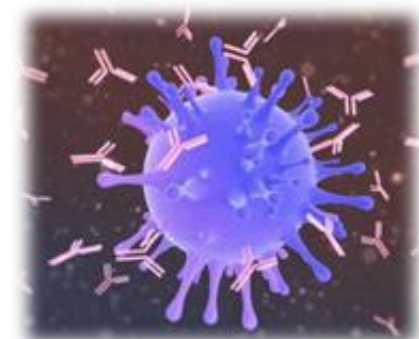
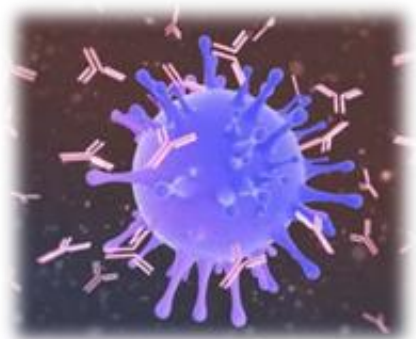
Prospective analysis

Starting April 2021

*Systematic screening of NSP swabs
positive for RT-PCR SARS-CoV-2 but
without any variants identification
using the commercially multiplex test*

*Screening of NSP swabs with
clinically suspected COVID-19 but
negative for RT-PCR SARS-CoV-2*

*Screening of NSP swabs in some
specified clusters*



Ion AmpliSeq SARS-CoV-2 Research Panel

One assay surveying complete SARS-CoV-2 genome for epidemiological investigation

1-10 ng total RNA

1 ng cDNA input per pool

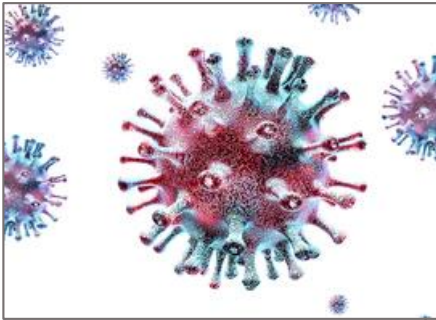


Nucleic acid
sample input



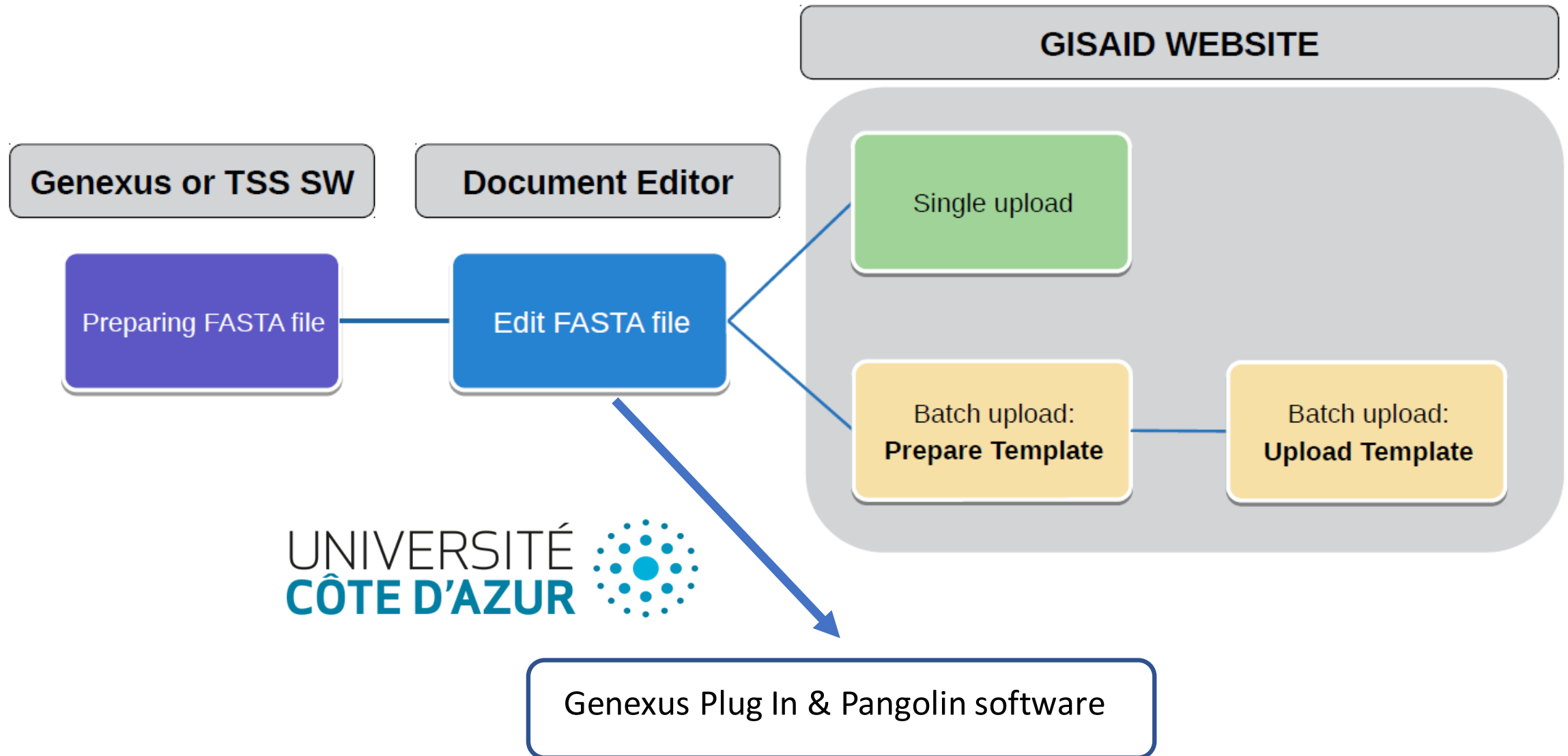
Automate cDNA synthesis library
prep, template prep, sequencing and
variant analysis

- **>99% coverage of SARS-CoV-2 genome (~30kb)**
- World leading amplification technology: AmpliSeq™
- Amplicon length range: 125-275 bp
- 237 amplicons specific to SARS-CoV-2 + 5 human expression controls
- All potential serotypes covered
- 2250 manual-preped, 600 Chef-automated libraries or 240 Genexus-automated libraries



Pool	No. Total Amplicons
1	247 (242 unique)*
2	

Data analyses for SARS COV 2 variants

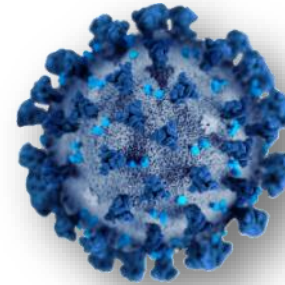


Identified variants in a retrospective analysis

Ongoing study conducted on the Ion Torrent Genexus system
total, 102 samples

Variants

B.1.160 (European)	64
B.1.1.77 (UK)	17
B.1.1.7 (UK)	8
B.1.241 (European)	3
B.23 (UK): 5 (4%)	2
B.1.1.2.41 (European)	1
B.1.1.2.21 (European)	1
B.1.596 (USA)	1
B.1.160.8 (Denmark)	1
A.19 (Burkina Faso)	1
B.1.411 (Sri Lanka)	1



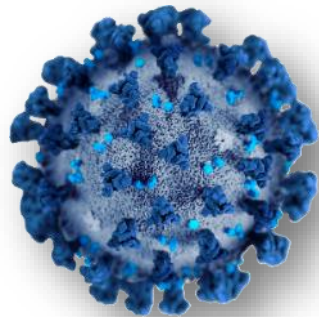
Variant classification



- **VOC (variants of concern)**
 - B.1.1.7 (UK)
 - B.1.351 (South Africa)
 - B.1.1.28.1 (Brazil)
 - B.1.617 (India)
- **VOI (variants under investigation)**
- **VUM (variant under monitoring)**

Comparative analyses obtained from matched nasopharyngeal swabs (NSP) and saliva samples

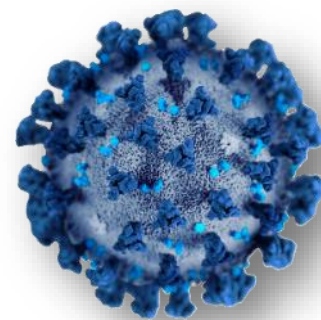
Samples	NSP 	Saliva 
1	B.1.160	B.1.160
2	B.1.1.7	B.1.1.7
3	B.1.160	B.1.160
4	B.1.1.2.41	B.1.1.2.41
5	A.19	A.19
6	B.1.411	B.1.411
7	B.1.596	B.1.596
8	B.1.177	B.1.177
9	B.1.160	B.1.160
10	B.1.160	failed
11	B.1.160	B.1.160
12	B.1.1.7	B.1.1.7
13	B.1.160	B.1.160
14	B.1.160	B.1.160



GENEXUS: More advantages, less constraints during the COVID-19 crisis

- Less contact between the health care workers during the manipulation (better biosafety)
- Easy to use
- Easy to analyse data
- Short turnaround time (24h)
- Workflow automation
- Flexible (integration of different workflows for genomic assessment of solid tumors too)
- High number of analyses (until 112 samples per week)
- High **sensitivity** of detection (able to detect variants even with a low viral load/high Ct)
- Panel design improvement Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – just launched





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Genomic evidence for reinfection with SARS-CoV-2: a case study

Richard L Tillett, Joel R Sevinsky, Paul D Hartley, Heather Kerwin, Natalie Crawford, Andrew Gorzalski, Chris Laverdure, Subhash C Verma, Cyprian C Rossetto, David Jackson, Megan J Farrell, Stephanie Van Hooser, Mark Pandori

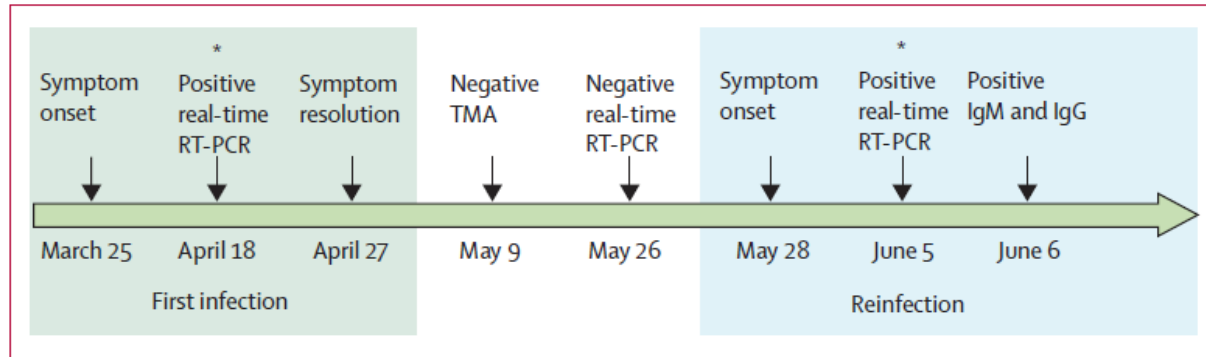


Figure 1: Timeline of symptom onset, molecular diagnosis, and sequencing of specimens
TMA=transcription-mediated amplification. *Sequenced specimens.

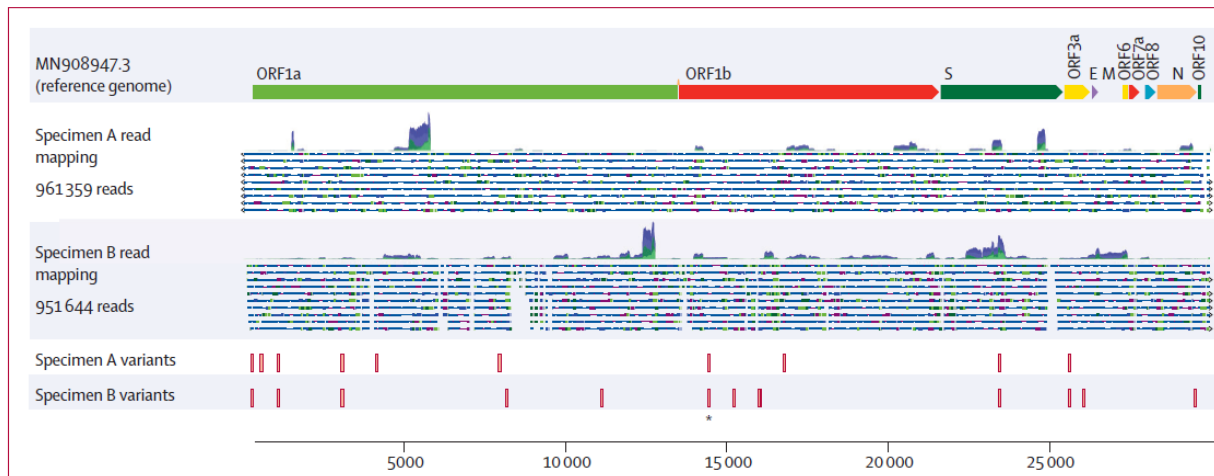


Figure 2: Variant mapping of specimens A and B against the reference genome
ORF1a and ORF1b encode replicase proteins. The other ORFs encode assembly proteins. ORF=open reading frame. S=spike. E=envelope. M=membrane. N=nucleocapsid. *Identifies variant 14 407 in specimen A and variants 14 407 and 14 408 in specimen B.



Emerging variants
to be permanently detected

**INDIA'S DOUBLE MUTANT
VARIANT 'CONCERN'**



A hospital worker monitors a person with COVID-19 in South Africa, where a new variant of SARS-CoV-2 has been identified.

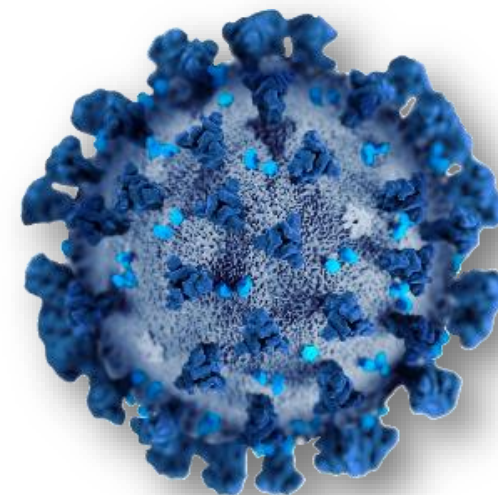
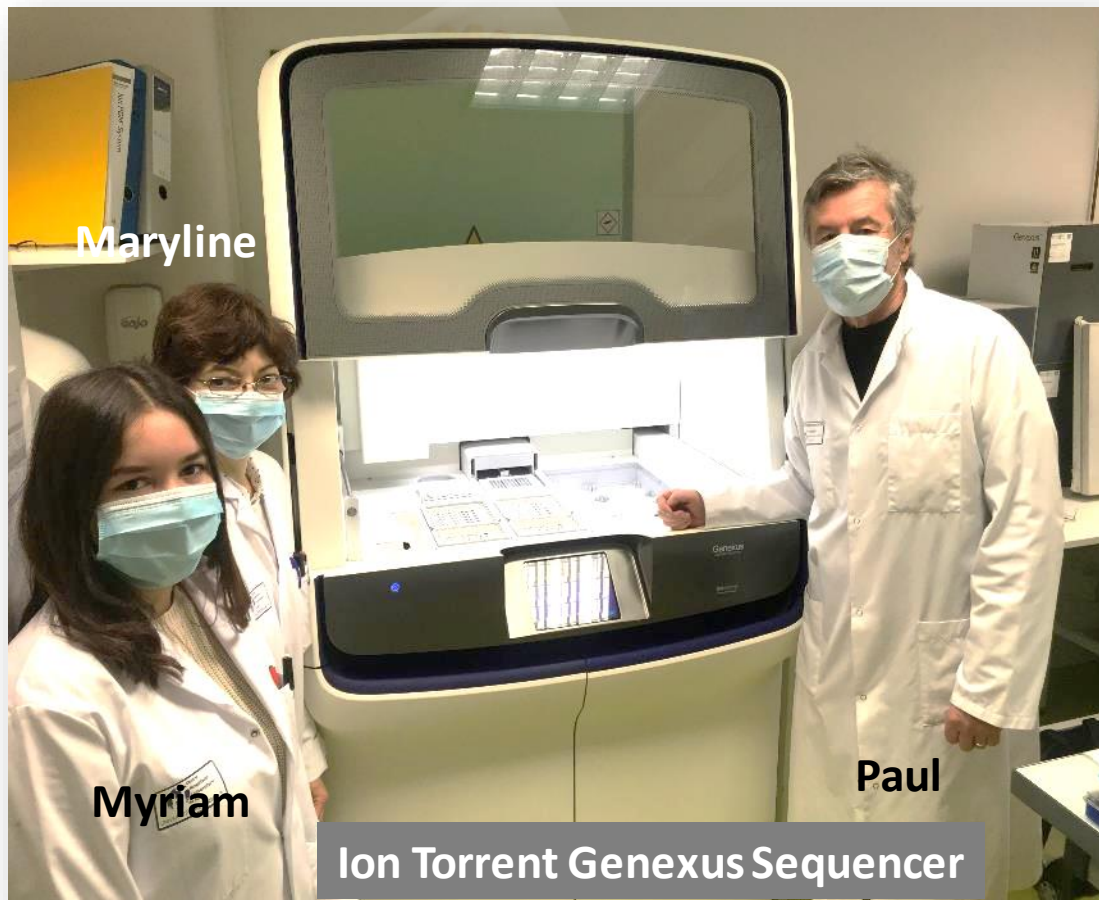
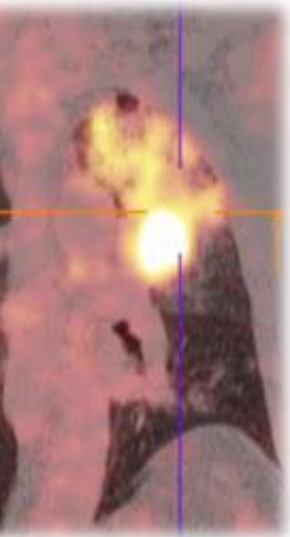
COULD NEW COVID VARIANTS UNDERMINE VACCINES? LABS SCRAMBLE TO FIND OUT

Researchers race to determine why lineages identified in Britain and South Africa spread so quickly, and whether vaccines will be less effective against them.





Integrating the activities in the same platform



Acknowledgments



LPCE Nice



@LPCE_Nice

