COVID-19: What is in the Box of Alternative Methods?

Conflict of Interest:
AxoSim, AstraZeneka, ATCC, Apple, Merck/Millipore, UL, ToxTrack
COVID-19 - so far a medical success story!

- Dec’19 First cases recognized
- 31 Dec’19 WHO informed
- 10 Jan’20 First PCR (UK)
- 11 Jan’20 Genome revealed (China)
- 17 Jan’20 WHO PCR diagnostic published (Germany)
- Mar’20 several antibody tests (EU)
- 1 May’20 FDA Emergency Use Authorization (not full approval) for drug Remdesivir

HIV 2018: 38 million infected, +1.7 million per year, 770,000 deaths
Jun’81 First cases;
Nov’83 1st WHO meeting
Mar’87 1st treatment
1996 1st home test kit
??? 1st vaccine

May’83 Virus identified
Mar’85 1st blood test
Aug’87 1st vaccine trial
1997 HAART therapy

Photo of cheering physicians (deleted for lack of copyright)

https://www.dreamstime.com/
Accuracy of COVID-19 diagnostics

PCR: Sensitivity 90+ % & Specificity 95+ %

SeroLogic: Sensitivity 88+ % & Specificity 96+ %

= BAC η 93%
Accuracy applied to testing

US: 40 cases per 10,000 citizens

The result of a **93% accurate test is positive**

Testing 10,000 people with this test will result in 37 real-positive but 700 false-positive:

Probability of COVID-19: \( \frac{37}{734} = 5\% \)

Of value only with clinical symptoms

Negative Case: Testing 10,000 will result in 9,300 real-negative but 3 false-negative:

Probability of no COVID-19: 99.9%

I WANT YOU TO GET A **COVID-19 TEST**
BIG WORRY

Are these 95% false-positives our “asymptomatic carriers”?
The medical challenge

Drugs

Vaccines

Fast, Please!

Photo of stethoscope under stress
(deleted for lack of copyright)
The Cochrane study register for COVID-19 listed (on 19 August 2020) more than 10,000 studies launched, with 2630 of them being interventional (https://covid-19.cochrane.org).

Already more than 160 vaccines for COVID-19 are under development (115 in April, Thanh et al. 2020).
Harnessing the power of novel animal-free test methods for the development of COVID-19 drugs and vaccines

Francois Busquet\(^1,2\) · Thomas Hartung\(^1,3\) · Giorgia Pallocca\(^1\@\) · Costanza Rovida\(^1\) · Marcel Leist\(^1,4\)

- Drug repurposing
- Target discovery
- Drug Efficacy
- Vaccine development
- Combination therapies
- Drug Safety
- Quality

*Photo of SARS-CoV-2 (deleted for lack of copyright)*
Evolution of Cell Culture - high-tech & business opportunity -

Marx et al., Biology-inspired micro-physiological system approaches to solve the prediction dilemma of substance testing using animals. ALTEX 2016, 33:272-321.


- Marx et al. 2016
Commercial human lung models

Remdesivir potently inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice

Andrés Pizzorno, Blandine Padey, Thomas Julien, Sophie Trouillet-Assant, Aurélien Traversier, Elisabeth Errazuriz-Cerda, Julien Fouret, Julia Dubois, Alexandre Gaynard, François-Xavier Lesecure, Victoria Dullière, Pauline Brun, Samuel Constant, Julien Poissy, Bruno Lina, Yazdan Yazdanpanah, Olivier Terrier, Manuel Rosa-Calatrava
doi: https://doi.org/10.1101/2020.04.27.064279

http://www.epithelix.com/products/mucilair

https://www.mattek.com/products/epiairway/

Characterization and treatment of SARS-CoV-2 in nasal and bronchial human airway epithelia

http://www.epithelix.com/products/smallair-hf
Organ-on-chip: LUNG

Human organs-on-chips as tools for repurposing approved drugs as potential influenza and COVID19 therapeutics in viral pandemics

Longlong Si, Haiqing Bai, Melissa Rodas, Wuji Cao, Crystal Yuri Oh, Amanda Jiang, Atiq Nurani, Danni Y. Zhu, Girija Goyal, Sarah E. Gilpin, Rachelle Prantil-Baun, Donald E. Ingber

doi: https://doi.org/10.1101/2020.04.13.039917

https://www.youtube.com/watch?v=52IL9egmyDw
Good Cell Culture Practice

Letter

Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0) – Draft for Stakeholder Discussion and Call for Action

David Pamies1, Marcel Leist2,3, Sandra Coecke4, Gerard Bowe4, Dave Allen5, Gerhard Gstraunthaler6, Anna Bal-Price4, Francesca Pistollato4, Rob deVries7,8, Thomas Hartung2,9 and Glyn Stacey10,11,12

GCCP 2.0
Draft published
• Stakeholder discussion
• Editor workshop
• Funding bodies

Register at: CAAT@jhu.edu

2016 - Testimony by Francis S Collins to Senate Labor, Health & Human Services Subcommittee, April 7, 2016
“I predict that, ten years from now, safety testing for newly developed drugs as well as assessment of the potential toxicity of numerous environmental exposures, will be largely carried out using human biochips ... [and] will mostly replace animal testing for drug toxicity and environmental sensing giving results that are more accurate, at lower cost, and with higher throughput.”
https://www.appropriations.senate.gov/hearings/hearing-on-fy2017-national-institutes-of-health-budget-request
Microphysiological Systems World Summit

New Orleans, 13-17 Dec 2021

Hosts:
Suzie Fitzpatrick, FDA
Thomas Hartung, Hopkins
Don Ingber, Harvard

~30 organizations
Scientific Advisory Board

Stay tuned!
OUR MINI-BRAIN PROJECT

▪ FROM SKIN OF DONORS, INDIVIDUAL STEM CELLS
▪ IN 3 MONTHS THOUSANDS OF IDENTICAL ORGANOIDs
▪ NEURONS COMMUNICATING
▪ SOME BRAIN FUNCTIONALITY
BrainSpheres

iPSCs → NPCs → BrainSpheres

NPC expansion

NPC single cell plating for 3D differentiation

Gyratory shaking 8 weeks

NANOG/OCT4/DAPI

NESTIN/FOXG1/DAPI

Pamies et al. ALTEX 2017, TAP 2018
Altered mental status  Stroke  Encephalopathy
Neurocognitive (dementia-like) syndrome  Encephalitis
Guillain-Barré syndrome
Acute cerebrovascular disease  Psychosis

Seen in 30-60% of patients
Virus infection

Microglia Increase Inflammatory Responses in iPSC-dervied Human BrainSpheres.

Cellina Monteiro Abreu¹, Lucio Gama², Susanne Krasemann³, Megan Chesnut⁴, Shelly Odwin-Dacosta⁵, Helena Hogberg⁶, Thomas Hartung⁷, David Pamies⁸

DENV-1

ZIKV-BR

Addition of micro-glia

Also:

HIV & JC virus

SARS-2-CoV

- Earlier neurotropic infection with Zika, Dengue, HIV and JC virus
- COVID-19 in Wuhan: 36% neurological symptoms, cases of virus encephalitis
- Other coronaviruses are neurotropic

William Bishai
Cynthia Korin Bullen

Our partners

... Abreu et al. Frontiers Microbiology, 2018; 9: 2766.
Coronavirus could infect human brain and replicate, US study shows

Johns Hopkins University research adds to concern about poorly understood neurological symptoms.
Short communication

Infectability of Human BrainSphere Neurons Suggests Neurotropism of SARS-CoV-2

Our DNT & DIT team

Cynthia Korin Bullen

Andrew Pekosz
Fig. 2: A small fraction of neuronal cells in BrainSpheres contains virus particles at 72 hpi
BrainSpheres infected with SARS-CoV-2 at MOI of 0.1 were analyzed at 72 hpi for M protein (red) expression by immunofluorescence (A) - (D). Neuronal marker MAP2 (green) was used to stain the neurons. Arrowheads indicate colocalization and presence of the virus in neurites. (E) and (F) BrainSpheres stained for SARS-CoV-2 spike protein (green). Images representative of five BrainSpheres. Nuclei are stained with Hoechst 33342 (blue). Scale bar 50 μm.
Transfer of organoids for more stringent washing
Presence of ACE2 receptor at all stages of mini-brain development

Some neural cells infected

Replication shown by PCR

Which cells? 
Pathology? 
Role of blood-brain-barrier? 
Disrupted neurodevelopment? 
Latent infection – neurodegeneration?

Just a first step....
19 August 2020:  
41,926 articles published  
+ self-published archives

Some people will have to read and condense them for us!  

Peer-review!!!  

Evidence-based approaches:  
Systematic review, QA scoring 

Photo of person
Overwhelmed by articles
(deleted for lack of copyright)
Anybody who can spell COVID-19 writes a grant or paper
Most are ill-equipped
Few get rich and these are mostly those selling the shuffles and sieves
Alternative methods are enabling technologies

Photo of SARS-CoV-2
(deleted for lack of copyright)