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The role of MPS in addressing the 3Rs in Zoonoses research involving protected species

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National Infection Service PHE

Microphysiological Systems (MPS):
Bridging Human and Animal Research
19th – 20th January 2021

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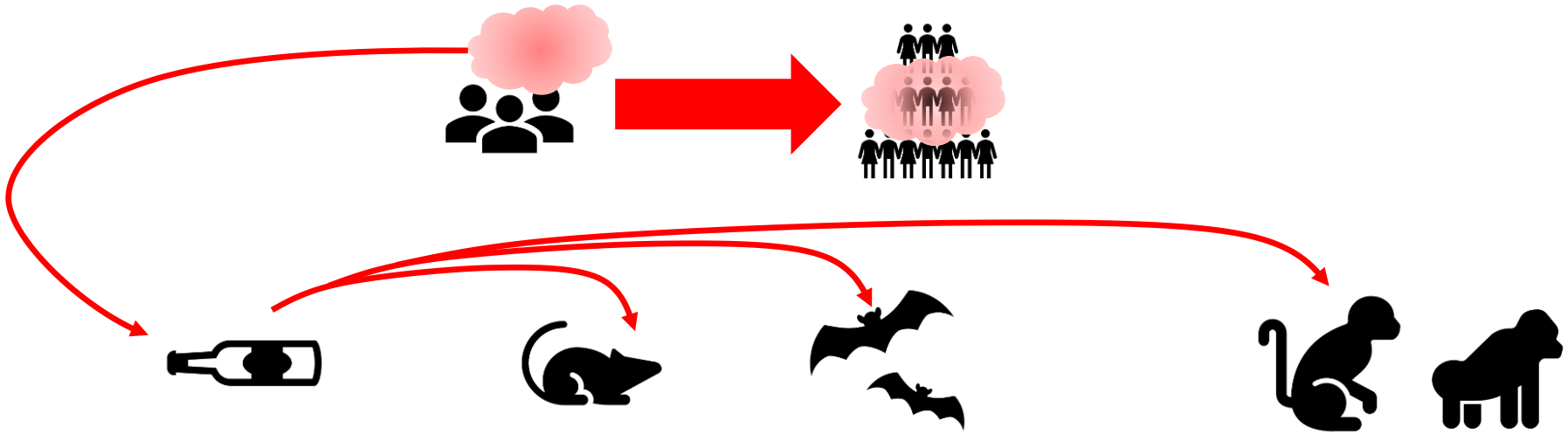
Overview



Traditional approach

Vero E6 | Rodent model | Exotic model | NHP model

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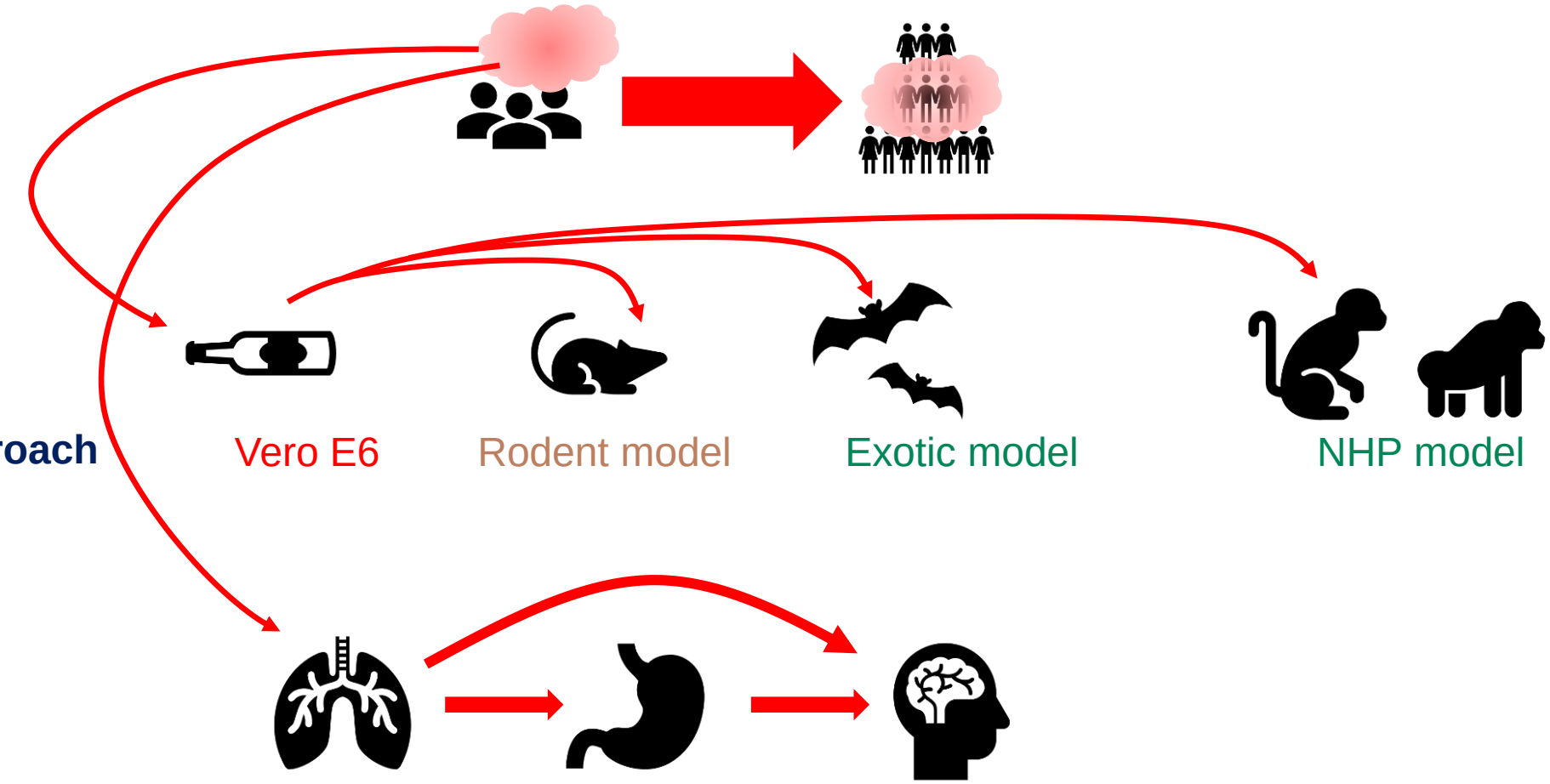
Overview



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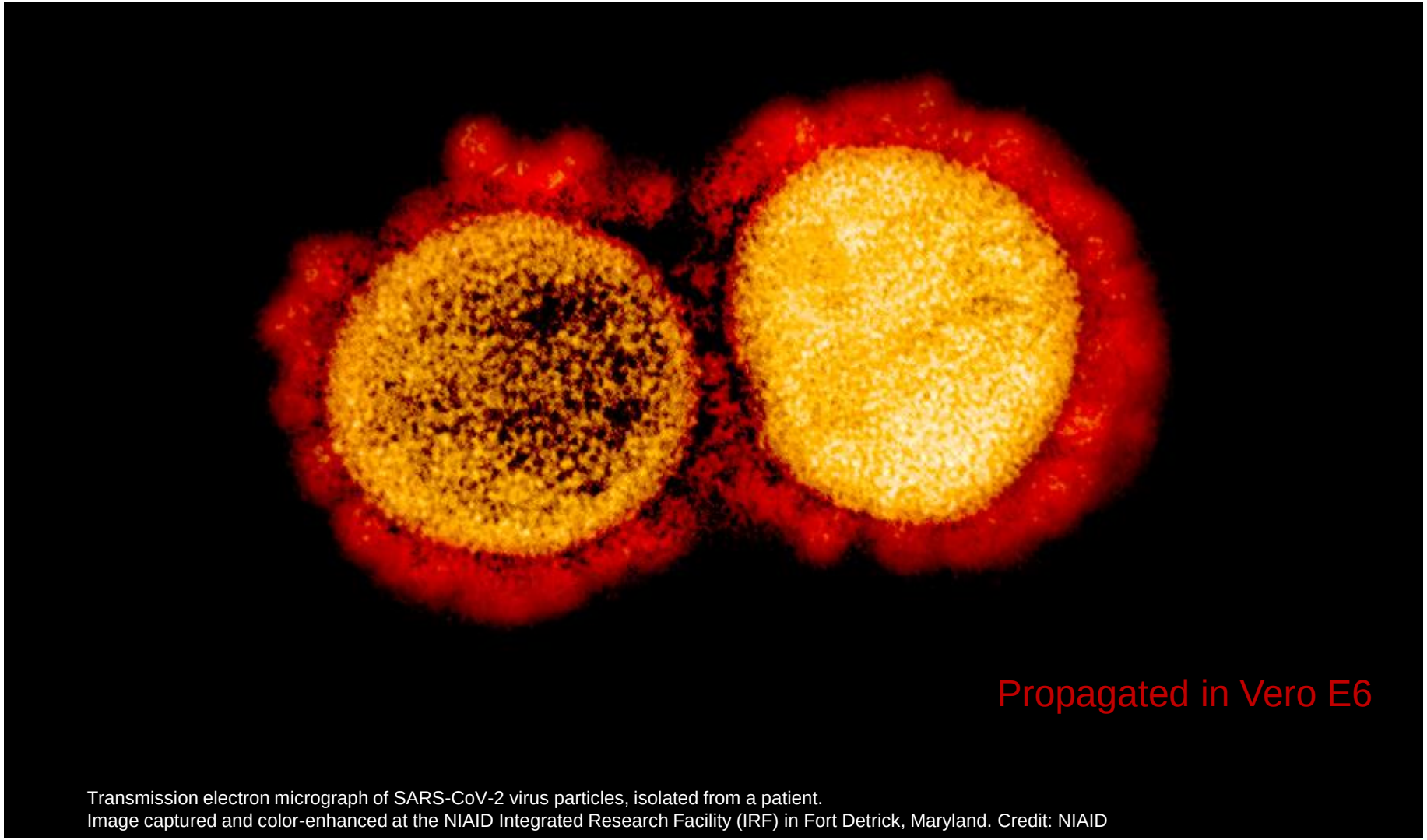
SARS-CoV-2



Transmission electron micrograph of SARS-CoV-2 virus particles, isolated from a patient.
Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID

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PHE's propagation in Vero/hSLAM

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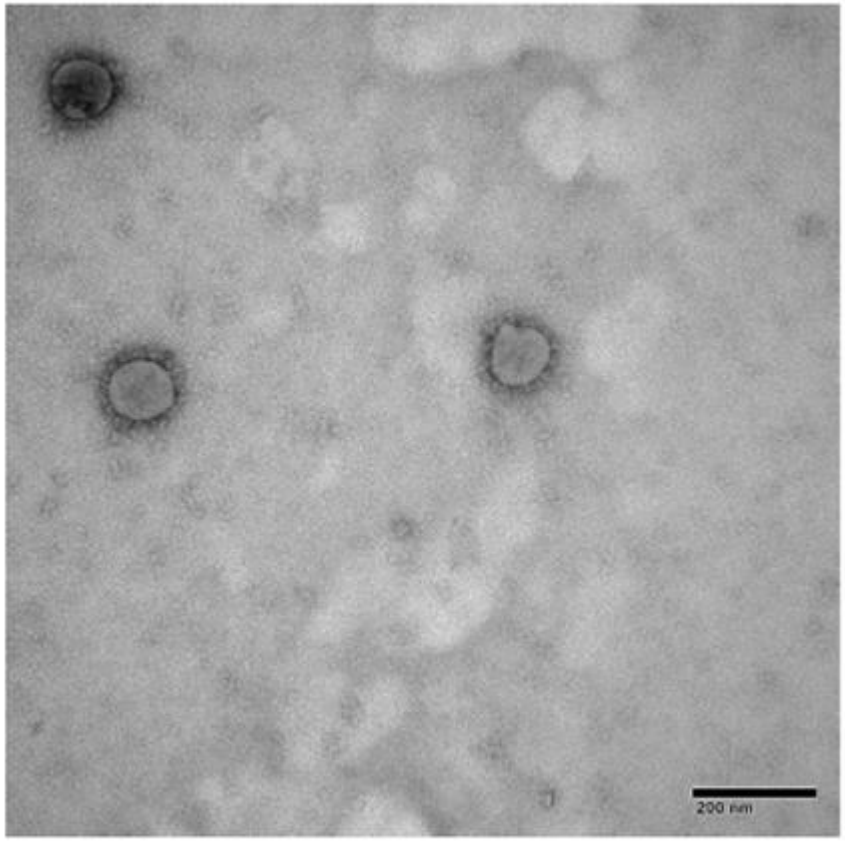
Passage History:

Passage 4: PHE Porton (ASL415) Grown from P3 into Vero/hSLAM cells (ECACC catalogue: 04091501) MOI of infection approx. 0.0005
Passage 3: PHE Porton (ASL406) Grown from P2 into Vero/hSLAM cells (ECACC catalogue: 04091501) MOI of infection approx. 0.0005
Passage 2: PHE Porton (ASL322) Grown from P1 into Vero/hSLAM cells (ECACC catalogue: 04091501)
Passage 1: Australian lab details (ASL320) Donation from the Peter Doherty Institute; 500µl vial labelled "2019-nCoV (Victoria/1/2020) P1 Ex-Vero 30/1/2020". Isolated from patient on Vero/hSLAM cells.
Passage 0: Human origin Nasopharangeal swab isolated in Vero/hSLAM. See Caly, L *et al.* 2020. Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. *Med J Aust.* doi: 10.5694/mja2.50569. From 58 year-old male from Wuhan, China felt unwell on returning to Melbourne on 19th Jan 2020. Developed fever on 20th Jan and a cough with sputum production on 23rd Jan. Admitted to Monash Medical Centre 24th Jan. No contact with live food markets, hospitals or known COVID-19 cases. Patient recovered and was discharged from hospital on 7th February 2020. Sequence published: GenBank Accession MT007544.1, GISAID EPI_ISL_406844 **Medical History:** Diabetes Mellitus – type 2 Previous smoker – ceased 4 years prior.

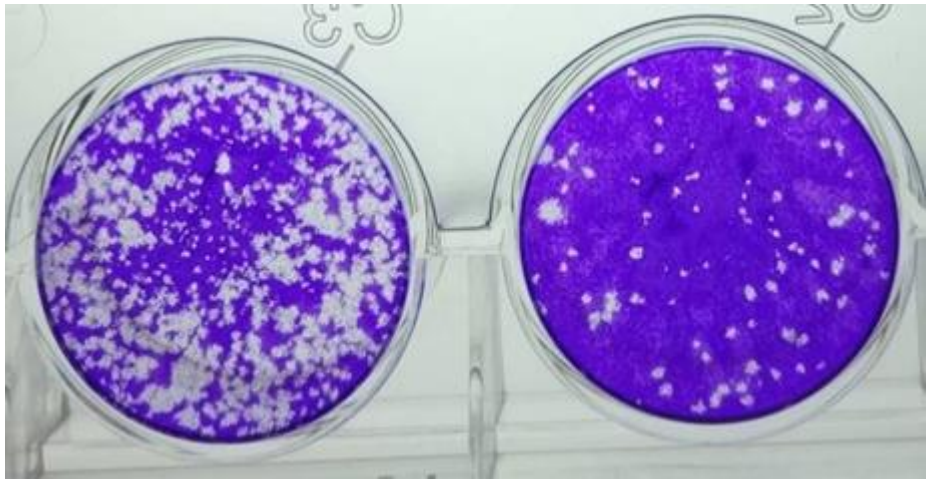
Transmission Electron Micrograph of material (negative staining)



Credit: Dohery Inst.,
Dr. K. Bewley,
MIG team and
PHE EM unit

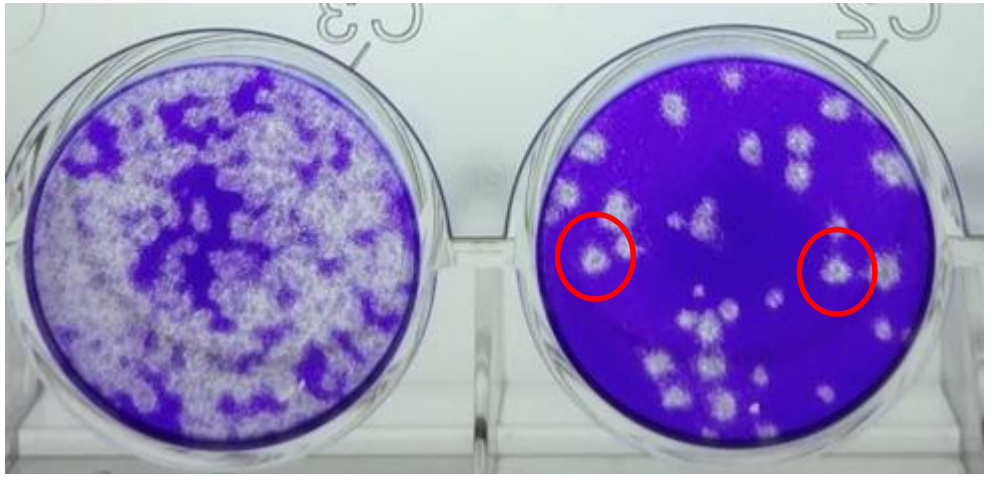
SARS-CoV-2 Vic 01 plaque morphology differences

Dilution of virus in Vero E6s



Small, well defined colonies
Better for titration purposes

Dilution of virus in Vero/hSLAMs



Large doughnut morphology

Plasmid vector (pCXN2) Neomycin resistance gene and
Expression plasmid (pcAG-hSLAM) encoding
human signalling lymphocyte activation molecule

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Vero E6 can increase ΔFCS

- Problem identified in February 2020
- WHO working group set up September
- Subject of WHO models call 8th October 2020
- Rapid communication being drafted
- There is a risk in propagation in Vero E6
- Mitigate by sequencing or Vero/hSLAMs
- Human cells may be beneficial – ongoing studies

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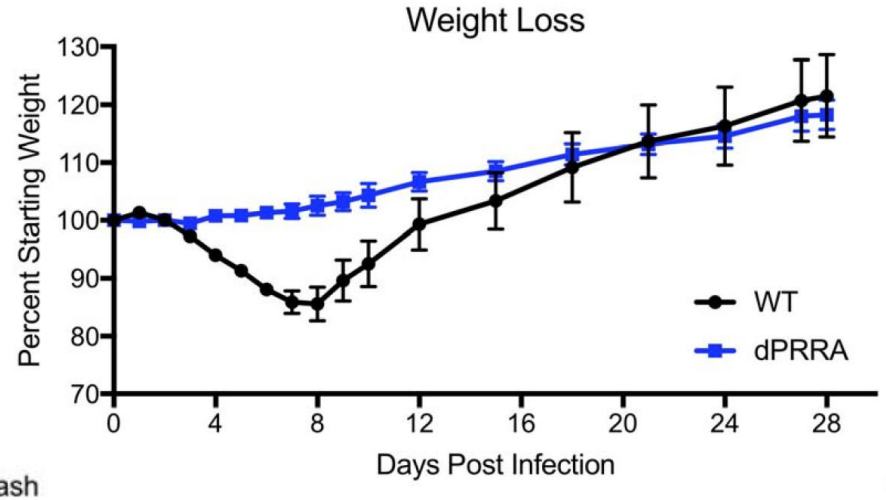
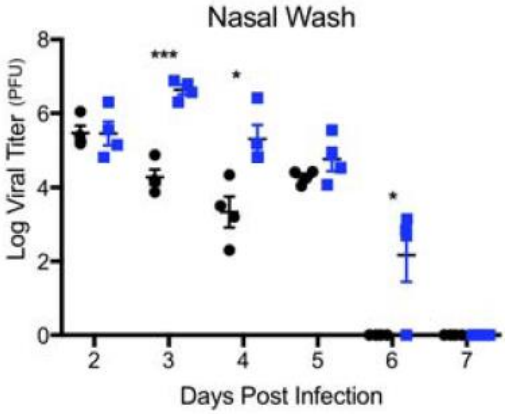
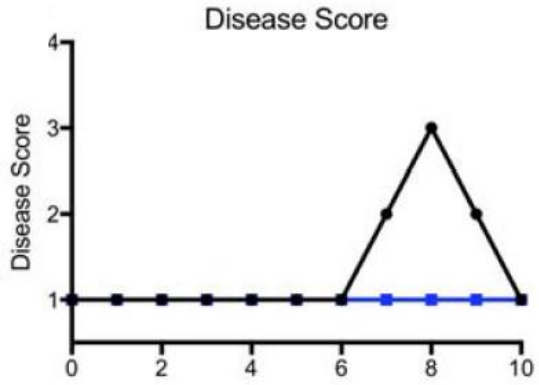
ΔFCS less disease in hamsters







Johnson *et al* doi 2020.08.26.268854

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Vero cells – a closer look

Available globally

Easy to grow

Widely used for viral propagation

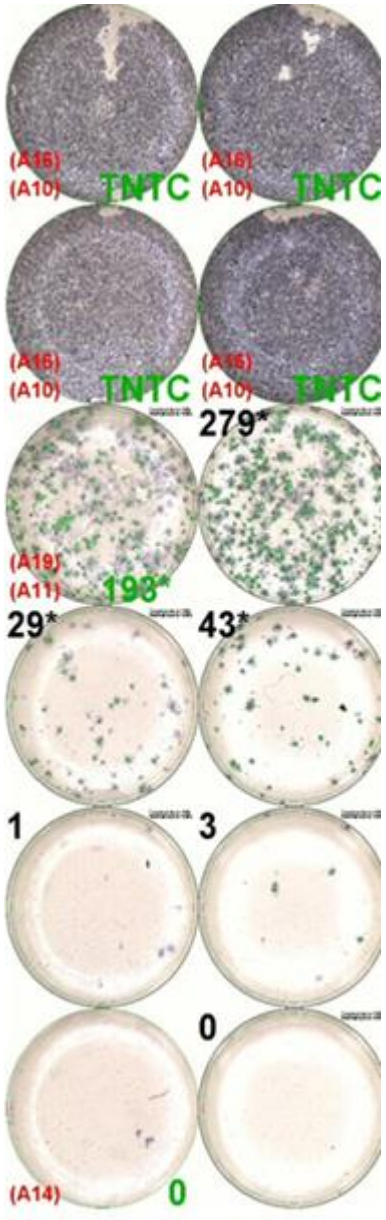
Widely used for plaque assays due to CPE

Chromosome 12 deletion

Loss of ability to produce Type 1 IFN

Loss of some element of regulation

Kidney cell origin



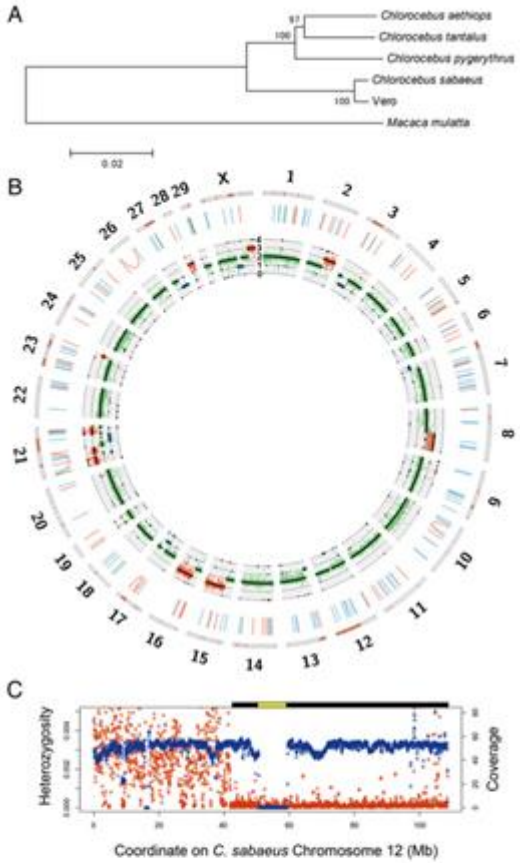


Figure 2. Genome landscape of the Vero genome. (A) Phylogeny of mitochondrial genomes of the Vero cell line, four *Chlorocebus* species, and *Macaca mulatta*. Bootstrap values with 1,000 replications were shown upon the branches. (B) Circos plot of the Vero cell genome. The orange bars in the outermost rectangles represent LOH regions. The blue, green, and orange lines in the middle layer show deletions, duplications, and inversions larger than 1 kb, respectively. The innermost plot shows the coverage of paired-end reads and expected ploidy (black lines). The blue, green, and orange dots represent the coverage values in 1×, 2×, and 3× regions, respectively. (C) The large deletion and LOH regions on *Chlorocebus sabaeus* chromosome 12. The red and blue points represent average heterozygosity (the number of heterozygous SNVs per bp) and genome coverage of paired-end reads in 1-Mb-size windows, respectively. The predicted homozygous deletion regions and LOH regions are shown as yellow and black bars on the plot area, respectively.

Osada *et al.*, DNA Res 2014

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Cells for propagation?

Cell types	Advantage	Disadvantage
Vero E6	Widely used, easy to grow	Can permit drift and shift
Vero/hSLAM	Restrict drift and shift Good titres at harvest	Not widely used + addition
Calu3	Restrict shift and drift	Lower titre, slow growth
CaCo3	Restrict shift and drift	Lower titre, slow growth
Human “tissue”	Better simulation of *in vivo*	Not easy to set up

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Which cells are best for infection models?

Cell types	Advantage	Disadvantage
Vero E6	Widely used	Not OK for HCQ or other May give wrong outcome
Vero/hSLAM	Reduces shift and drift	Not widely used + addition
Calu3	Reduces shift and drift	Not widely used
CaCo3	Reduces shift and drift	Not widely used
Human epi cells And MPSs	Very good simulation of *in vivo* May replace some *in vivo* in time	More demanding

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Preclinical evidence HCQ and Imatinib

Emerging preclinical evidence does not support broad use of hydroxychloroquine in COVID-19 patients
S. G. P. Funnell , W. E. Dowling, C. Muñoz-Fontela, P.-S. Gsell, D. E. Ingber, G. A. Hamilton, L. Delang , J. Rocha-Pereira, S. Kaptein, K. H. Dallmeier, J. Neyts K. Rosenke, E. de Wit , H. Feldmann, P. Maisonnasse , R. Le Grand , M. B. Frieman & C. M. Coleman
Nature Communications
doi: https://doi.org/10.1038/s41467-020-17907-w

Preclinical evaluation of Imatinib does not support its use as an antiviral drug against SARS-CoV-2
Franck Touret, Jean-Sélim Driouich, Maxime Cochin, Paul Rémi Petit, Magali Gilles, Karine Barthélémy, Grégory Moureau, Denis Malvy, Caroline Solas, Xavier de Lamballerie, Antoine Nougairède
doi: https://doi.org/10.1101/2020.11.17.386904

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Preclinical evidence on Hyroxychloroquine

Authors	Source	Test item	Test system	Dose	Antiviral	Symptomatic
Frieman *et al*	NIH	CQ HCQ	Vero cells + SARS-CoV		Yes	Mild effect
Frieman *et al*	NIH	CQ HCQ	Mice + MA SARS-CoV	1.0E+05 PFU	None	Yes
Kaptein *et al*	KU Leuven	HCQ	Hamster + SARS-CoV-2	2.0E+06 $TCID_{50}$	None	None
Rosenke *et al*	RML NIAID	HCQ	Hamster + SARS-CoV-2	1.0E+04 $TCID_{50}$	None	None
Massonaise *et al*	Inserm	HCQ	Cyno + SARS-CoV-2	1.0E+06 PFU	None	None
Minster *et al* Rosenke *et al*	RML NIAID	HCQ	Rhesus + SARS-CoV-2	2.8E+06 $TCID_{50}$	None	None
Ingber *et al.*	Wyss Inst	HCQ	Human respirator Emulate + Pseudovirus	-	None	None
Massonaise *et al*	INSERM	HCQ	Human respiratory Mucilair™ + SARS-CoV-2	6.3E+06 to 4.3E+07 $TCID_{50}$	None	None

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CaCo3	Reduces shift and drift	Not widely used
Human epi cells And MPSs	Very good simulation of *in vivo* May replace some *in vivo* in time	More demanding*

* New technology is “democratising” MPSs

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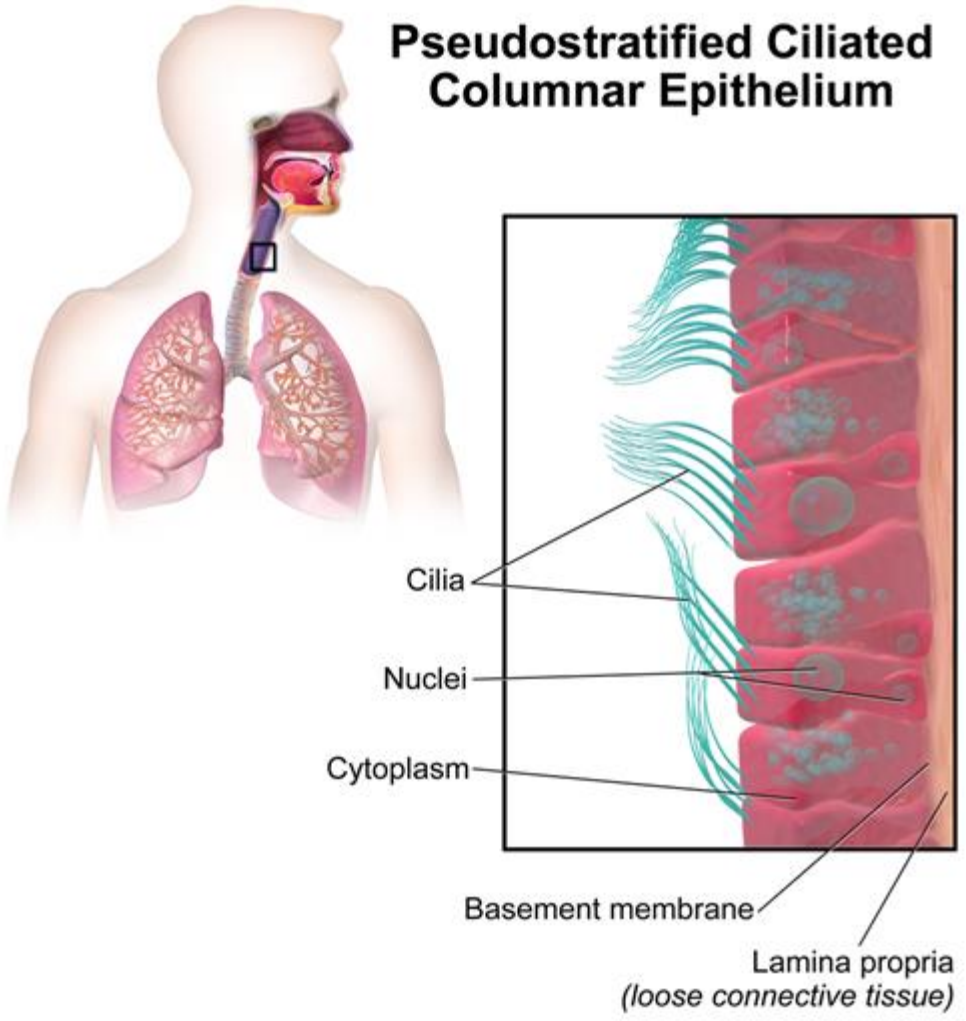
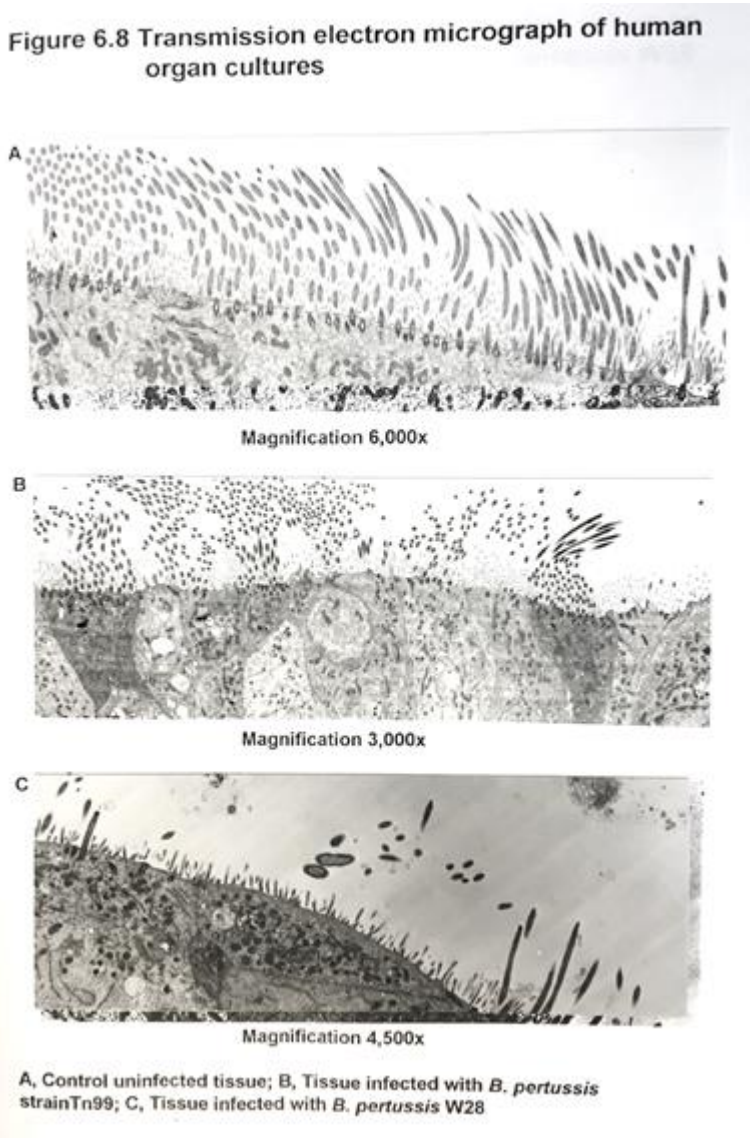
URT "Shag pile carpet" surface





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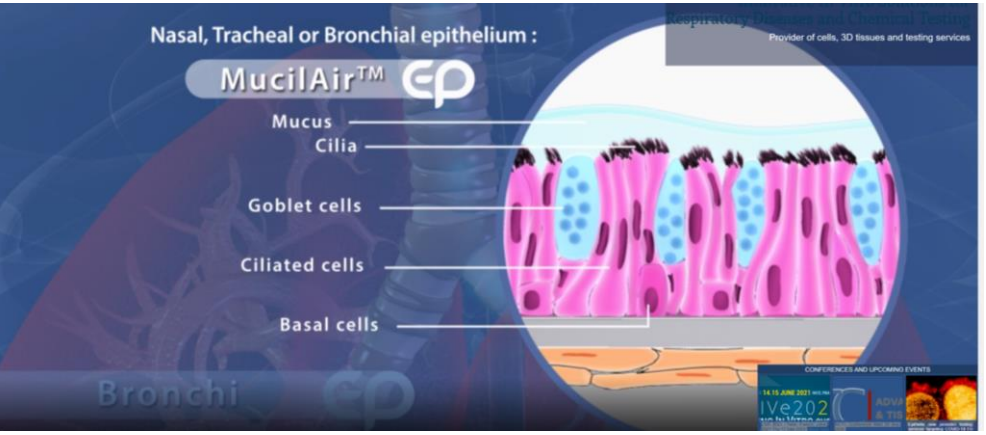
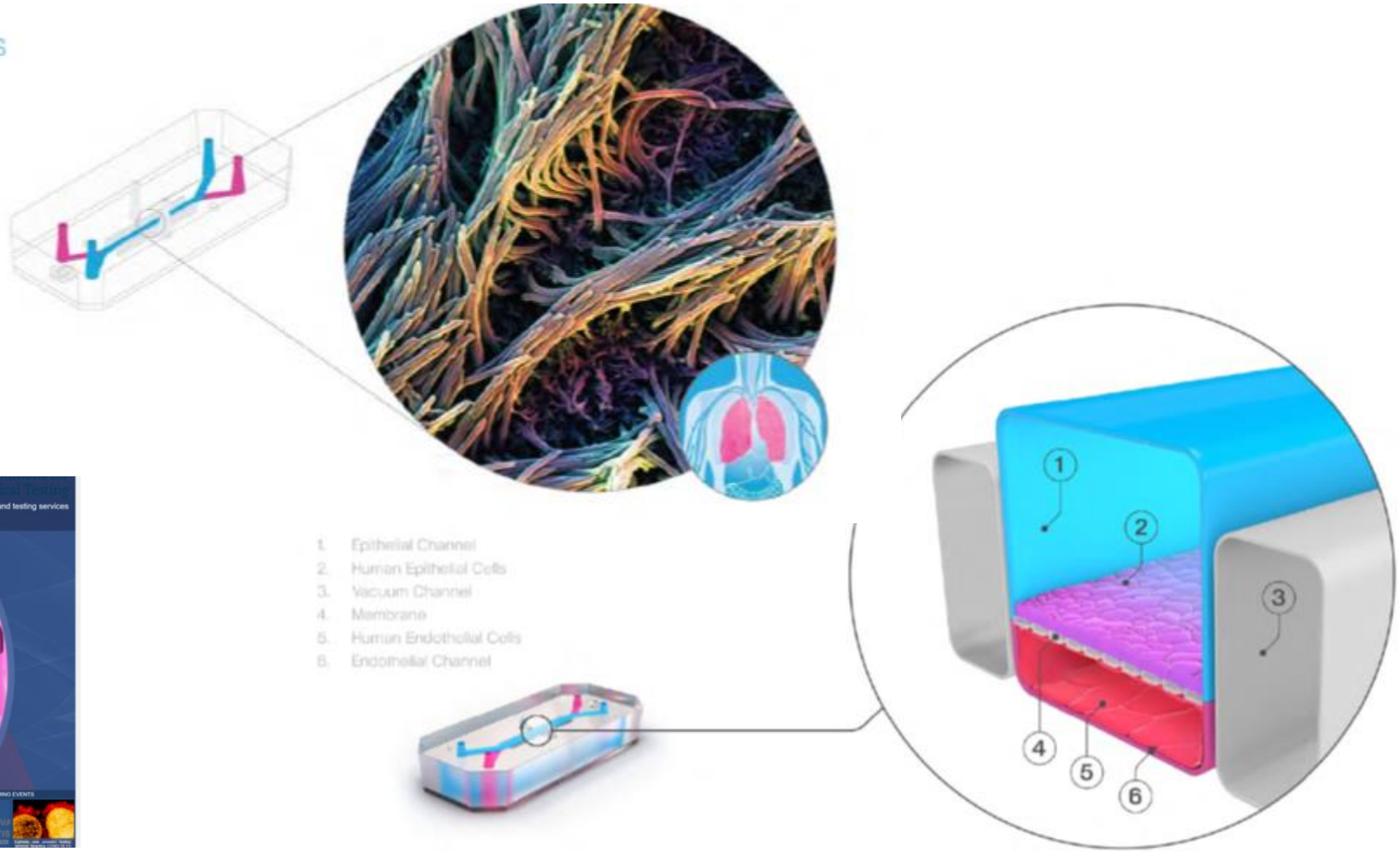
Microfluids (Organ on a chip)

In vitro infection using human respiratory organ-on-a-chip technology

- Extracellular matrix and cell interactions
- Cell shape and cytoarchitecture
- Tissue-tissue interactions
- Mechanical forces
- Dynamic system – flow
- Resident or circulating immune cells





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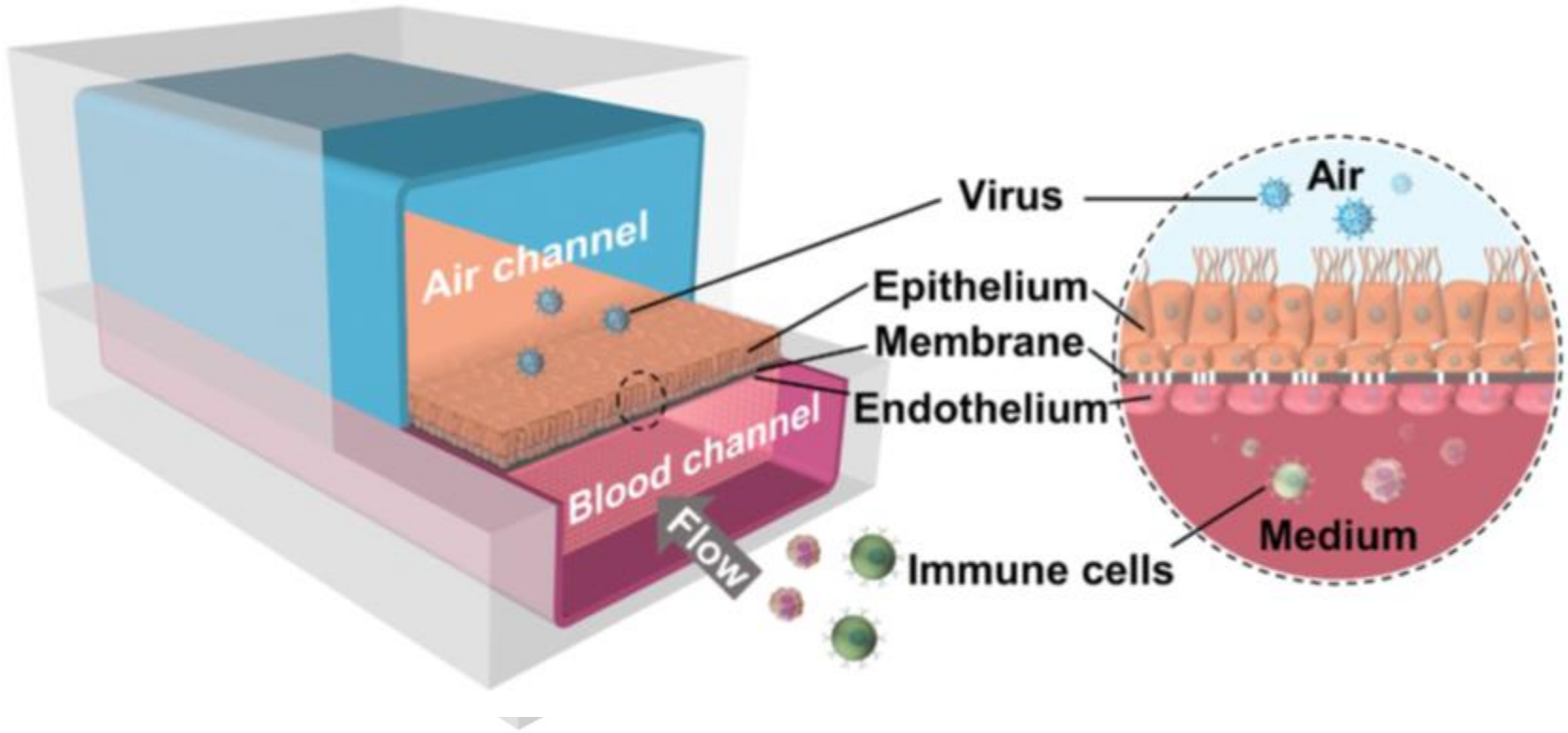
Refinement of *in vitro* models of infection



Longlong *et al*., 2020; https://doi.org/10.1101/2020.04.13.039917

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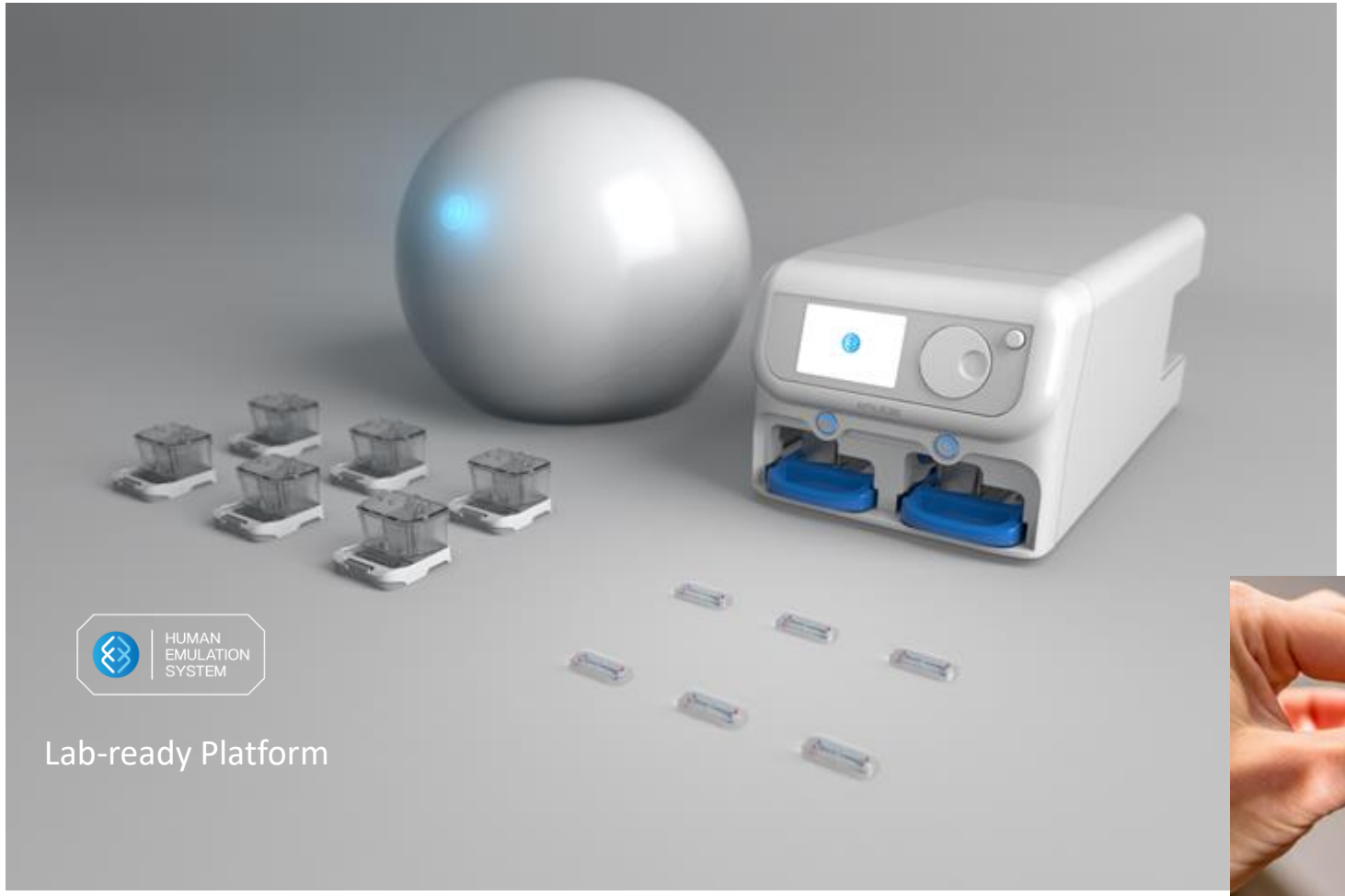
“Lab ready” solutions



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Overview of SARS-CoV-2 modelling landscape

Host species	Disease	Main metric
Mouse	Mild	Viral shedding
Hamster	Moderate	Viral shedding and weight loss
Ferret	Mild	Viral shedding (URT focus) pathology
Mink	Moderate	Unknown
Pigs	Resistant	Not applicable
Cats	Moderate	Viral shedding
Marmosets	Mild	Viral shedding
African green NHP	Mild	Viral shedding (extra), imaging, LRT pathology
Rhesus macaque	Mild	Viral shedding, imaging, LRT pathology
Cynomolgus macaque	Mild	Viral shedding, imaging, LRT pathology
Complex organ culture	Mild	Viral shedding, LRT pathology

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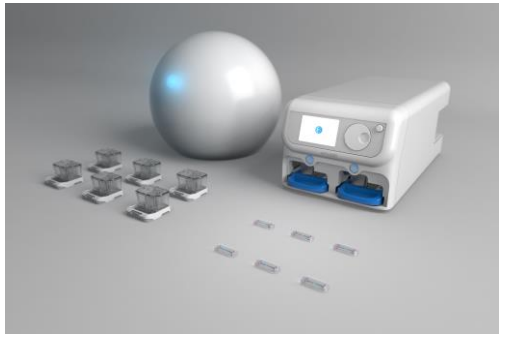
Resource requirements

Traditional	Microphysiological systems
Breeding staff and facility	Initial source materials
Screening and surveillance	Frozen seed stocks
Considerable Ethics burden	Limited ethical requirements
Planning	Fast set up
Surge capacity limitations	Scale up possible
Infrastructure	Capital equipment
Large ABSL3 facility	BSL3 laboratory
Large Staff resource need	Smaller staff requirement



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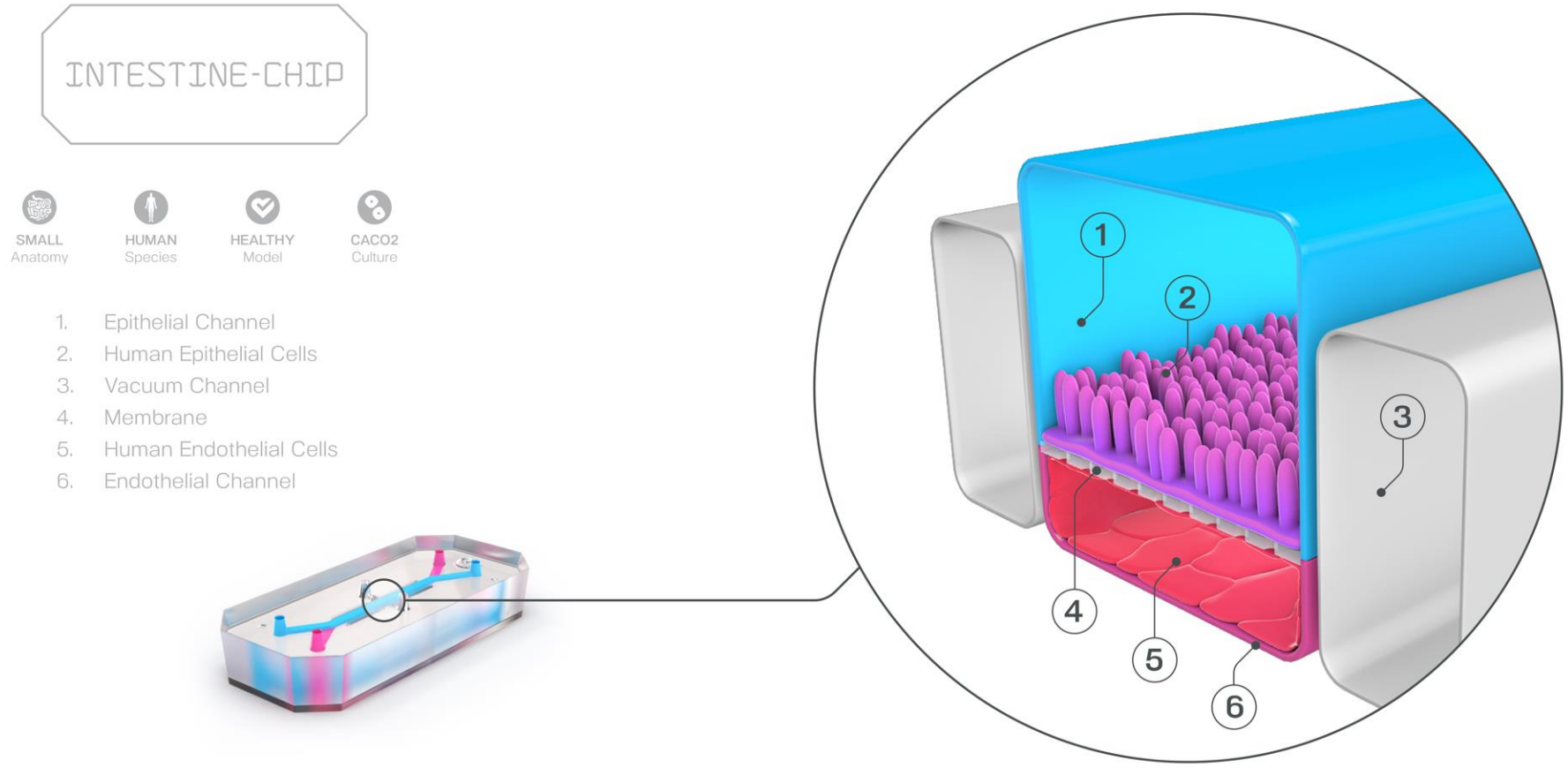
Human intestine chip: epithelium+CaCO2



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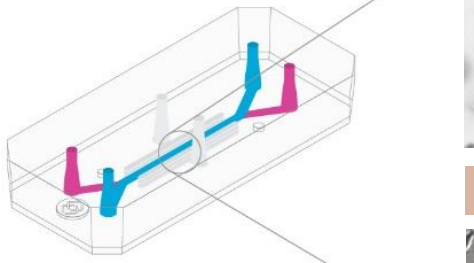
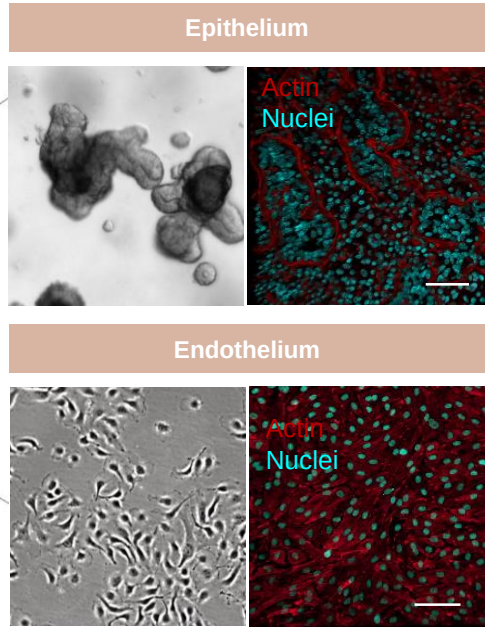
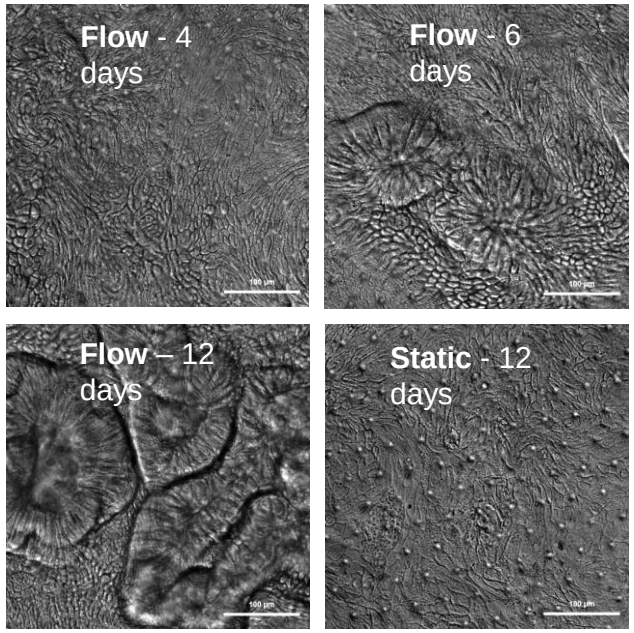
Primary Intestine-Chip: Human endothelium/epithelium







Top channel – Intestinal epithelial cells derived from intestinal organoids

Bottom channel – Intestinal microvascular endothelium (HIMEC)

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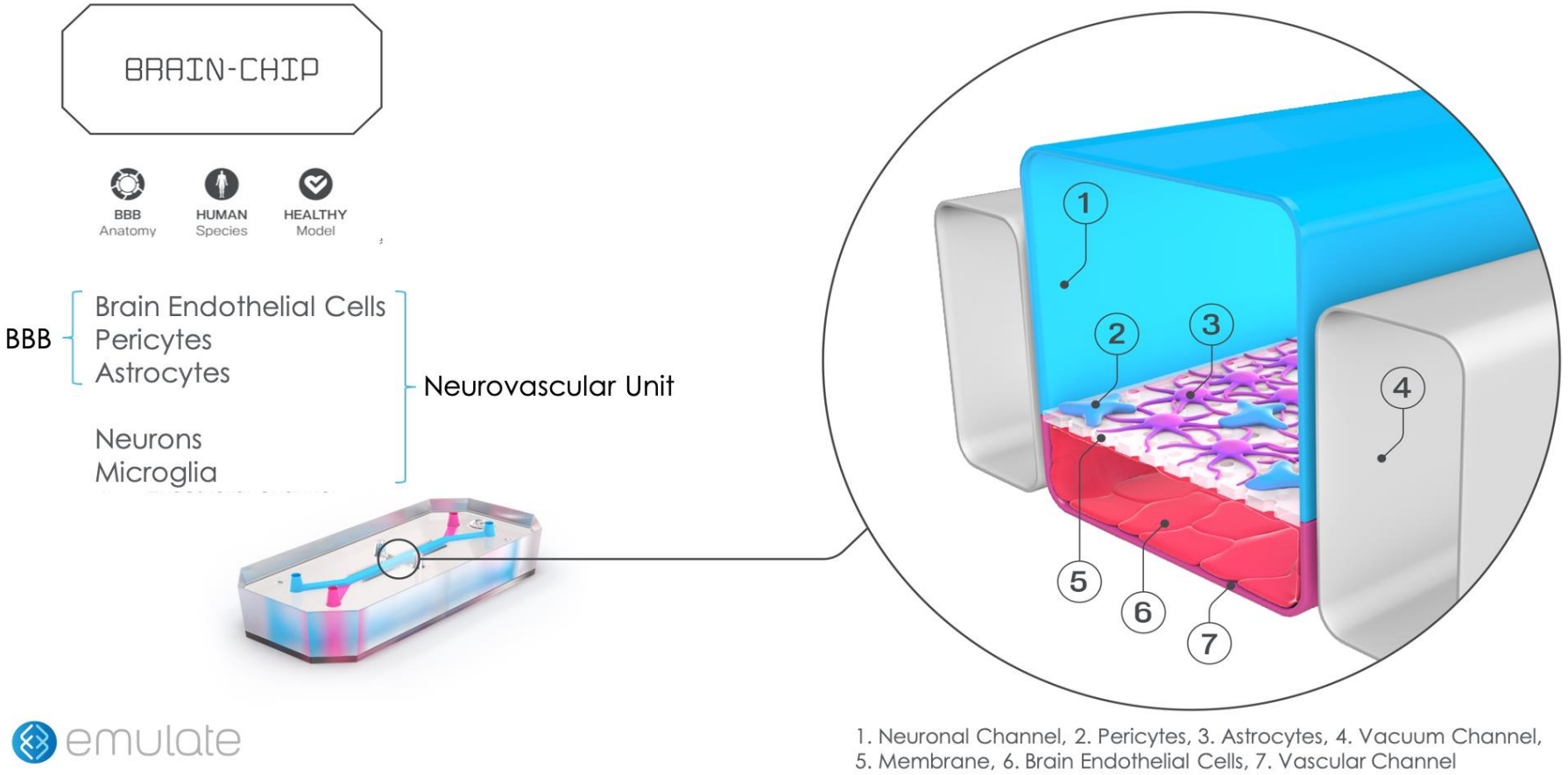
Human Brain chip: endothelial/pericyte and astrocyte chip



1. Neuronal Channel, 2. Pericytes, 3. Astrocytes, 4. Vacuum Channel,
5. Membrane, 6. Brain Endothelial Cells, 7. Vascular Channel

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Unanswered questions

Pre-clinical

Is there an unchartered animal reservoir?
How do MPS outcomes compare with *in vivo* outcomes?
Do new variants vary in their species specificity?
Can mice, bats, rhesus and cynos be infected with B.1.1.7?
Can mice, bats, rhesus and cynos be infected with “new variants”?
Are there organ specific tropisms & differences across species?

Clinical

What is the mechanism of severe disease onset?
Is pre-existing N protein immunity a risk factor?
What causes the cytokine storm?
Why are males more susceptible to severe disease?
Is E protein an important virulence factor?
Could E protein ion channel inhibitors be therapeutic?

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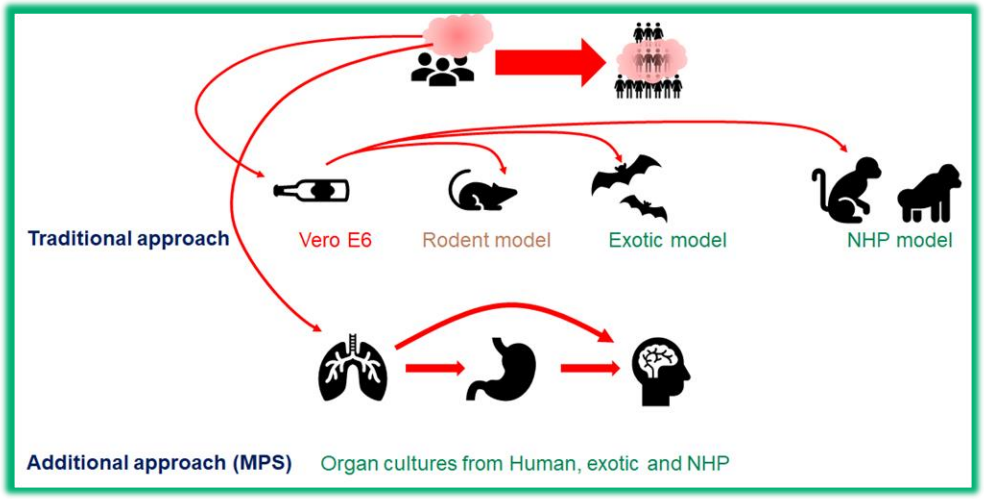
Summary



Cell “lines” can deceive

Pre-screening could be refined

MPS Technology has enabled multiple tissue differentiation *in vitro*

MPSs support *in vivo* outcomes

Vero E6 assessments should/can be replaced

Multispecies (eg Bat and primate) susceptibility and organ tropism possible *in vitro*

Animal usage could be reduced