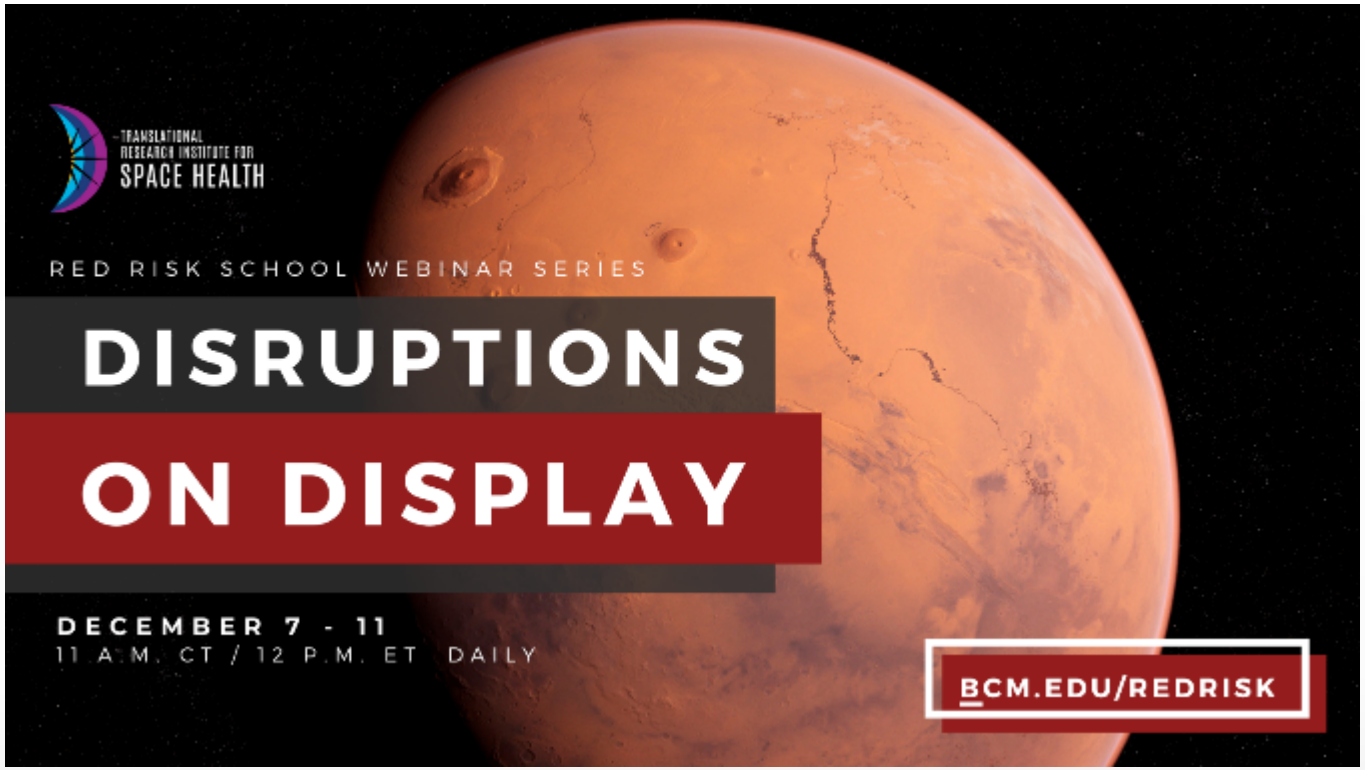


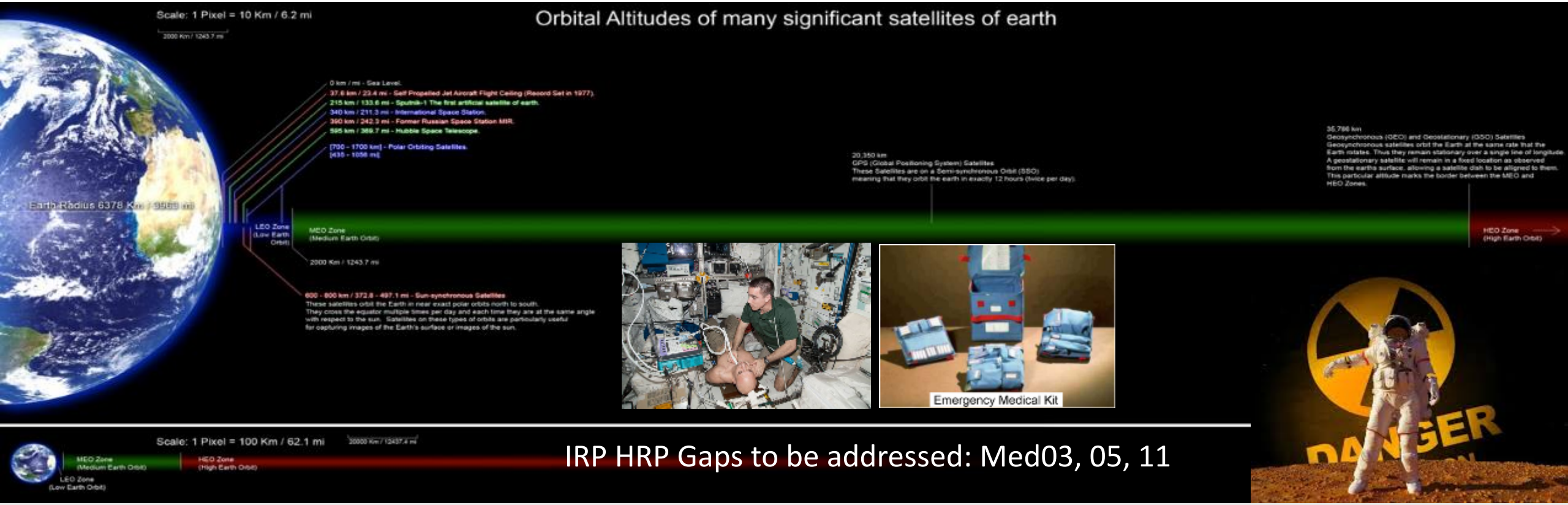
THE NEW TEST STRIP

Frederic Zenhausern, Ph.D., MBA, FNAI, FAIMBE
Director and professor

DISCLOSURES

Dr. Zenhausern disclosed that he is a Consultant for Wren Laboratories LLC and founder of Whitespace Enterprise Corporation

NASA New Era: *Travel Beyond Low Earth Orbit*



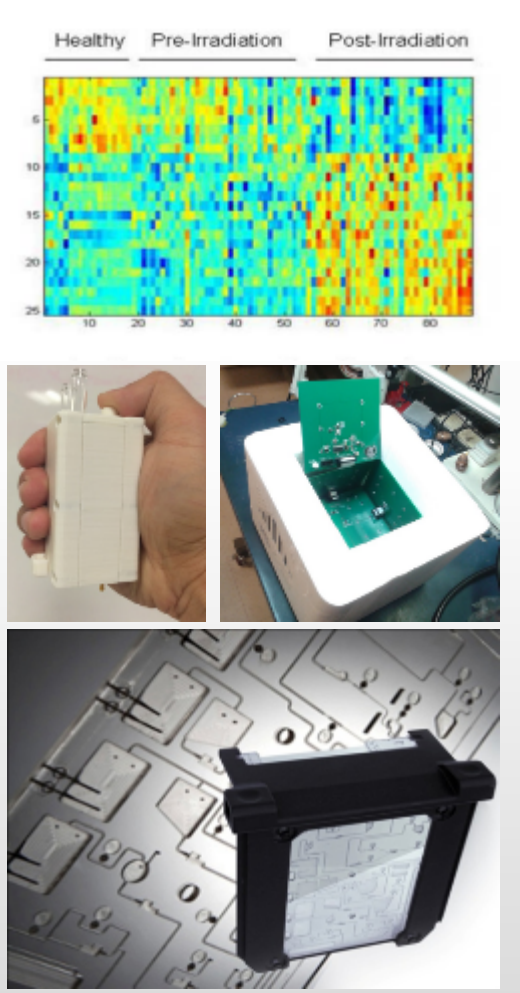
IRP HRP Gaps to be addressed: Med03, 05, 11

ISS Distance from Earth: 230 Miles
Moon Distance from Earth: 238,900 Miles
Mars Distance from Earth: ~140,000,000 Miles

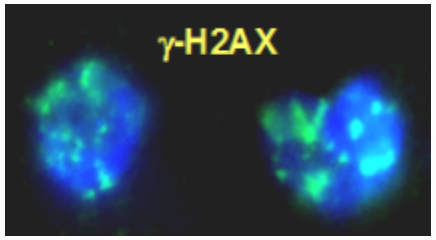
NASA Twins Study
Scott and Mark Kelly
12 months mission

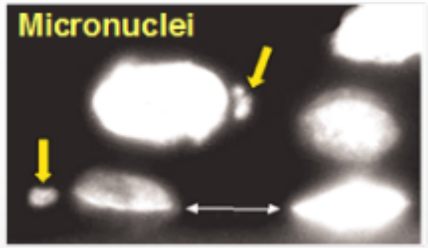
Assay Platforms for Radiation Exposure

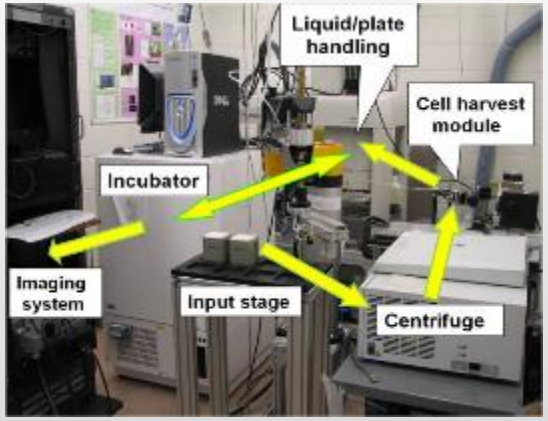
GENOMICS



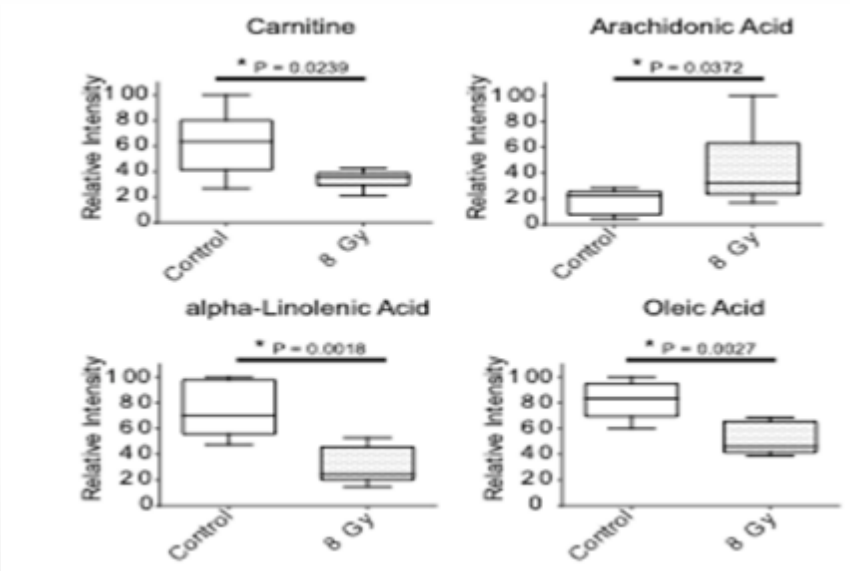
CYTOGENETICS

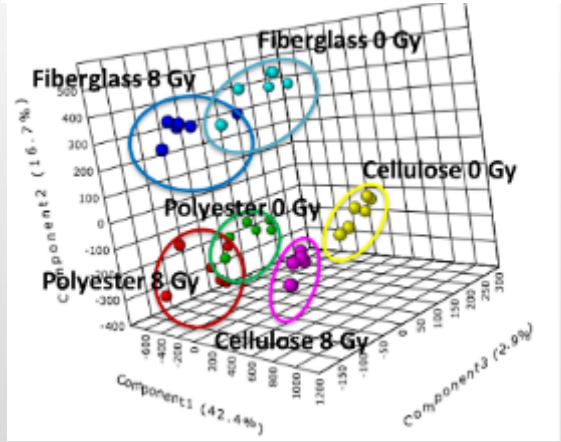






METABOLOMICS





NIAID CMCR 5U19AI067773-13

Blood, Saliva, urine…

BARDA HHS0100201000001C

Microbiome & GCR Interactions

Presence of LGG bacteria modulates response for low and high LET irradiation

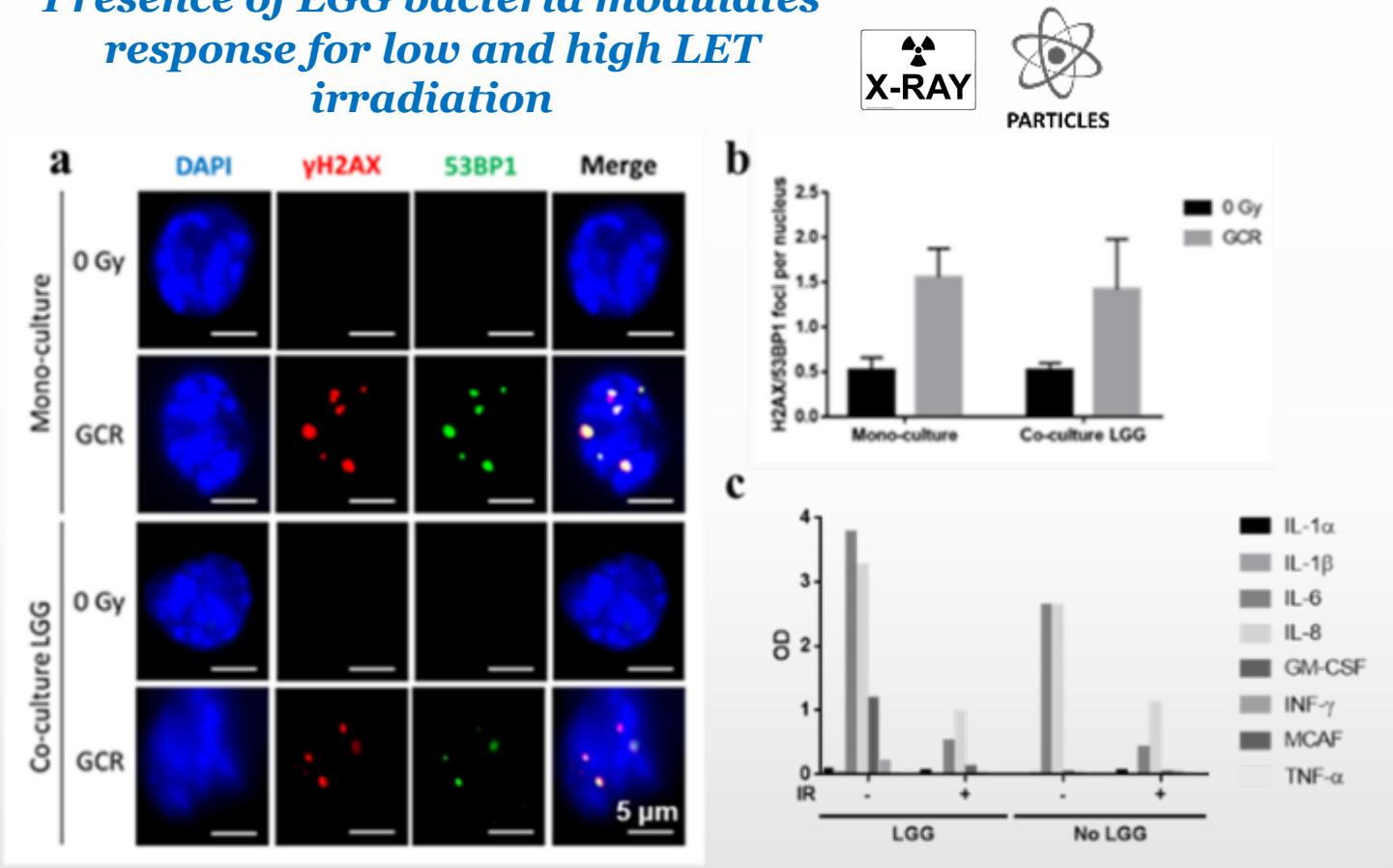
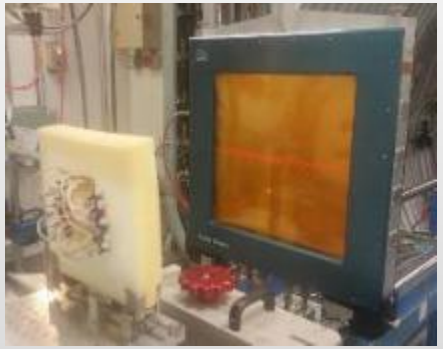
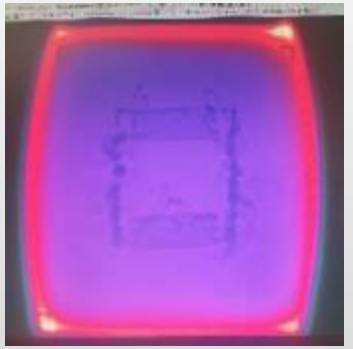


Figure 1. Preliminary data obtained at NSRL with HuMiX platform. Representative picture of Fhs 74 Int cells seeded on membrane in presence or not of LGG and exposed 7 days later to GCRSim (a). Quantification of γ-H2AX/53BP1 foci (n=100) (b). Profile of 8-cytokines mix from supernatant (c).

HuMiX device in NSRL beam

BROOKHAVEN NATIONAL LABORATORY





GCR = 0.75 Gy GCR H^+, ^4He, ^{16}O, ^{28}Si, ^{56}Fe
LGG = Lactobacillus rhamnosus

Challenges with Molecular Diagnostics Testing

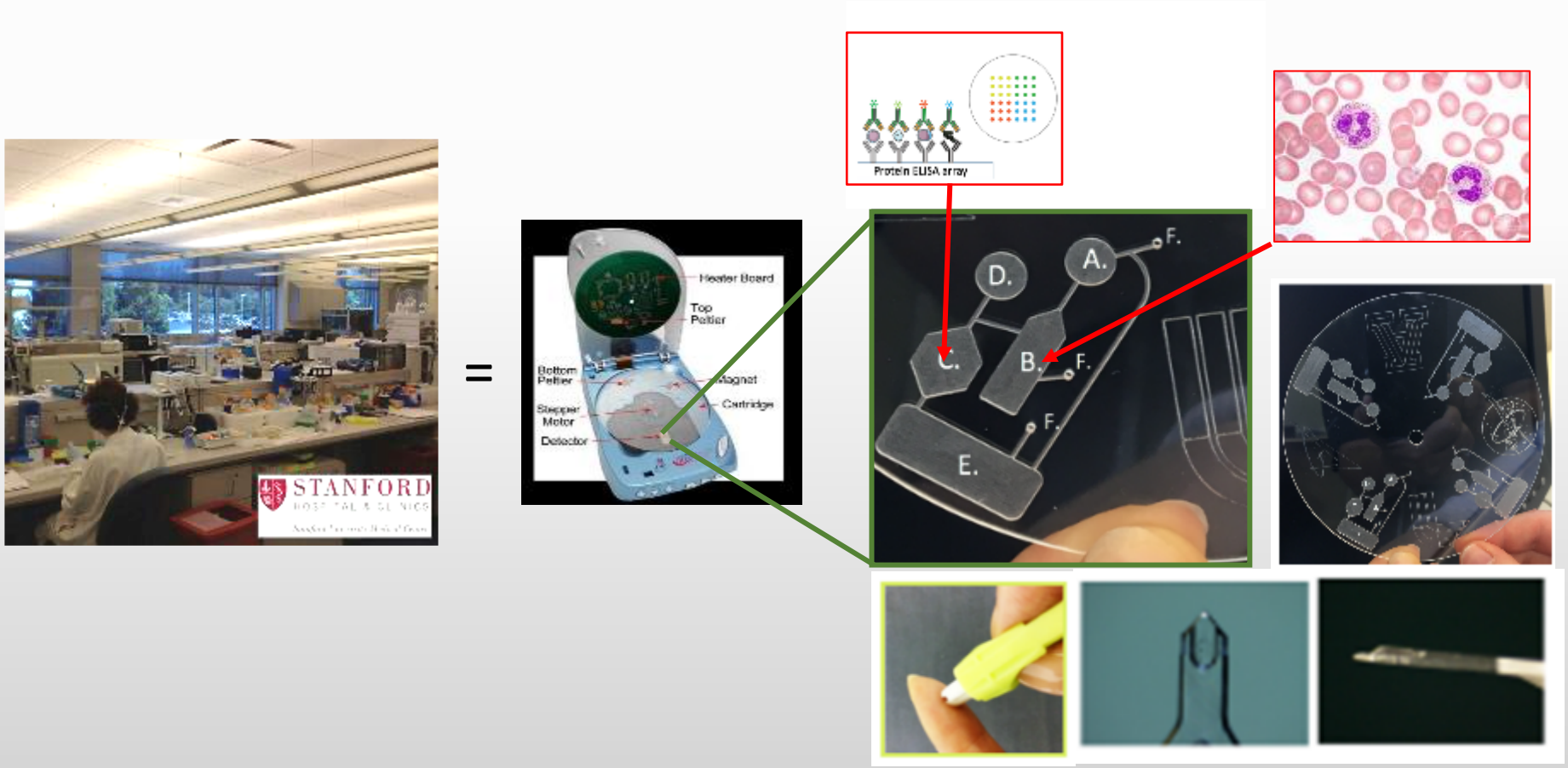
- Molecular tests do NOT translate readily into mobile point-of-care diagnostics

Multiplex
Throughput
Cost

Single-plex
One sample, low cost

Handheld Medical Diagnostic Device for Harsh Environments

Develop a prototype triage device that is inexpensive, rapid, easy-to-use and capable of blood cell differential counts and protein profiling for characterizing biological responses of radiation exposure.



Logistics, Validation and Deployment

VeriFast ™

Microgravity could offer new fluid behavior…

SPACE TANGO

CubeLab™

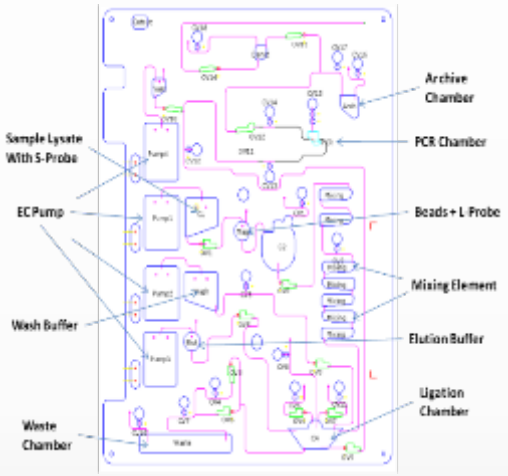
TRANSLATIONAL RESEARCH INSTITUTE FOR SPACE HEALTH

NASA

NEW SHEPARD / NS-10 TEST FLIGHT

The Current State of the Art:
Radiation-based gene expression

- 2019 CE approved
- The first gene expression biodosimetry test
- DNA/RNA based technology
- Laboratory platform



First…Vertical Flow Immunoassay (VFI) for the detection of *Burkholderia pseudomallei* surface capsular polysaccharide…

Contents lists available at ScienceDirect

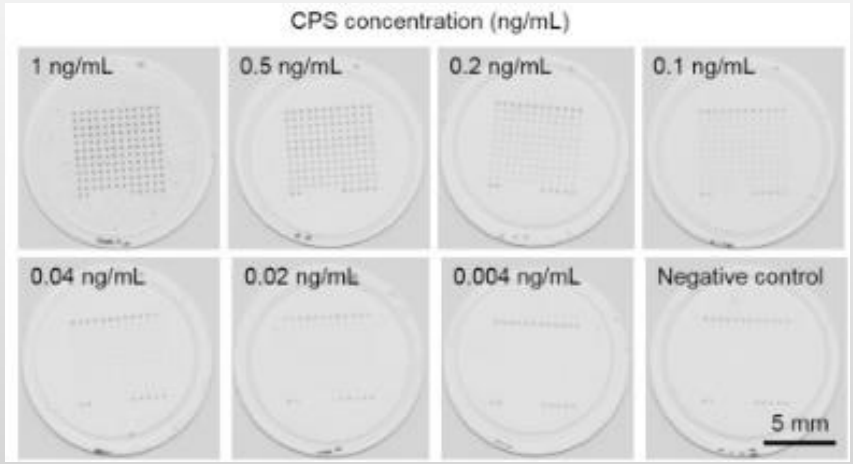
Talanta

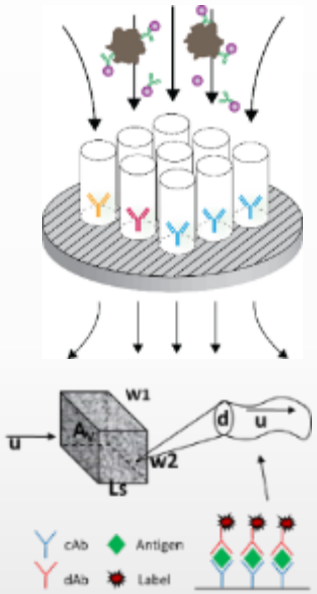
journal homepage: www.elsevier.com/locate/talanta

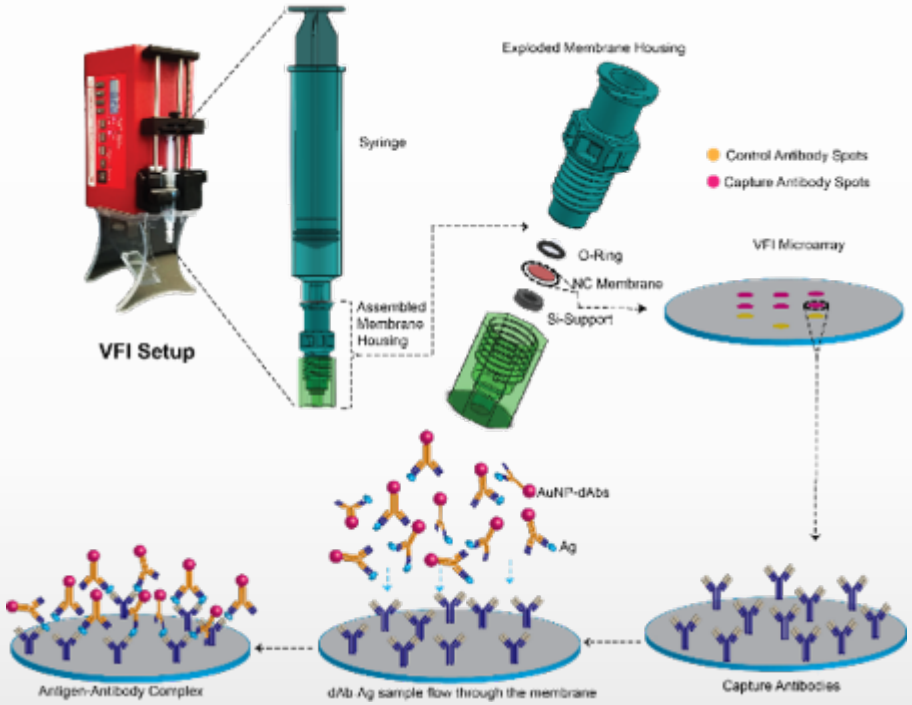
Paper-based Vertical Flow Immunoassay (VFI) for detection of bio-threat pathogens

Peng Chen[a], Marcellene Gates-Hollingsworth[b], Sujata Pandit[b], Anson Park[c], Douglas Montgomery[c], David AuCoin[b], Jian Gu[a,*], Frederic Zenhausern[a,*]

[a] Center for Applied Nanobioscience & Medicine, College of Medicine – Phoenix, University of Arizona, Phoenix, AZ, USA
[b] Department of Microbiology and Immunology, University of Nevada School of Medicine, Reno, NV, USA
[c] School of Computing, Informatics and Decision Systems Engineering, Arizona State University, Tempe, AZ, USA







u: flow speed L_s: sensing length
d: membrane pore size D: diffusivity

Diffusion time

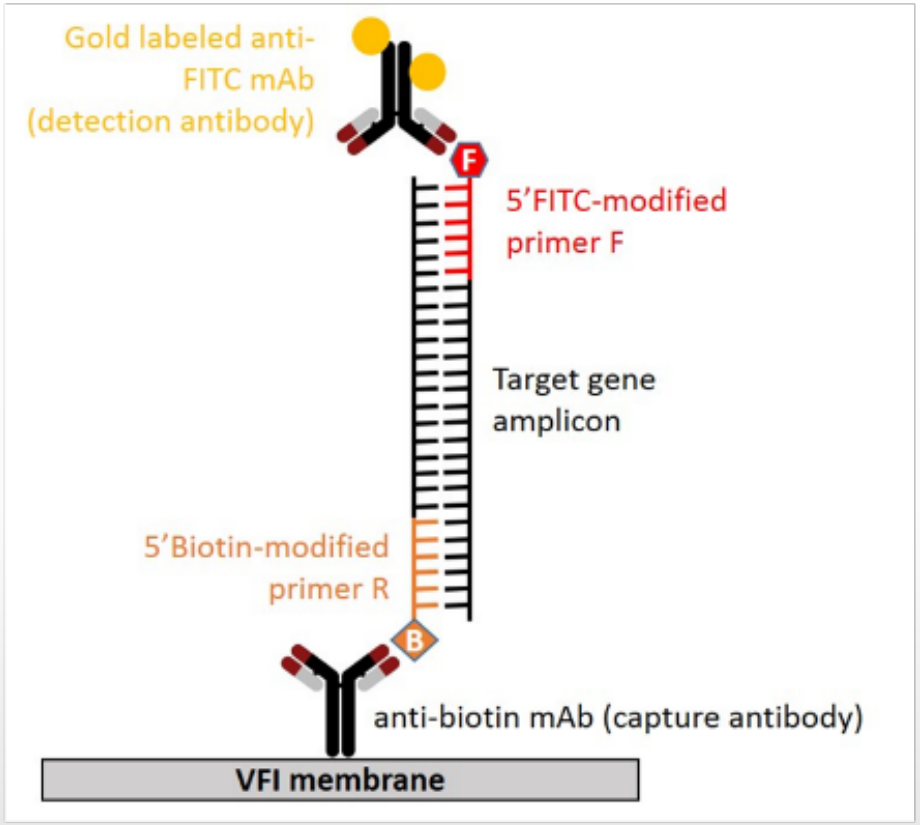
$$t_{dif} = \frac{d^2}{4D}$$

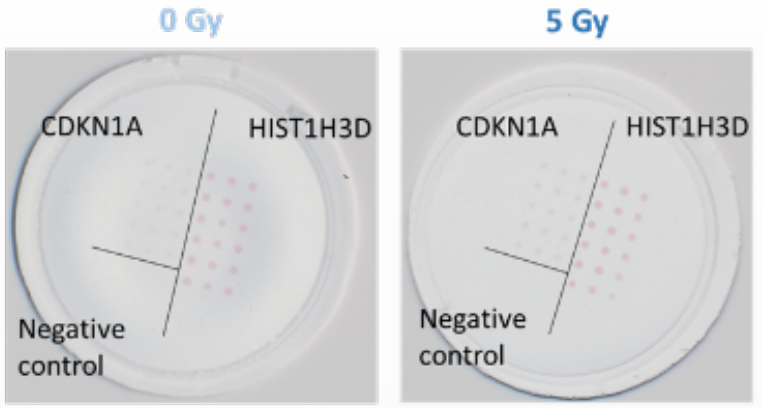
Resident time

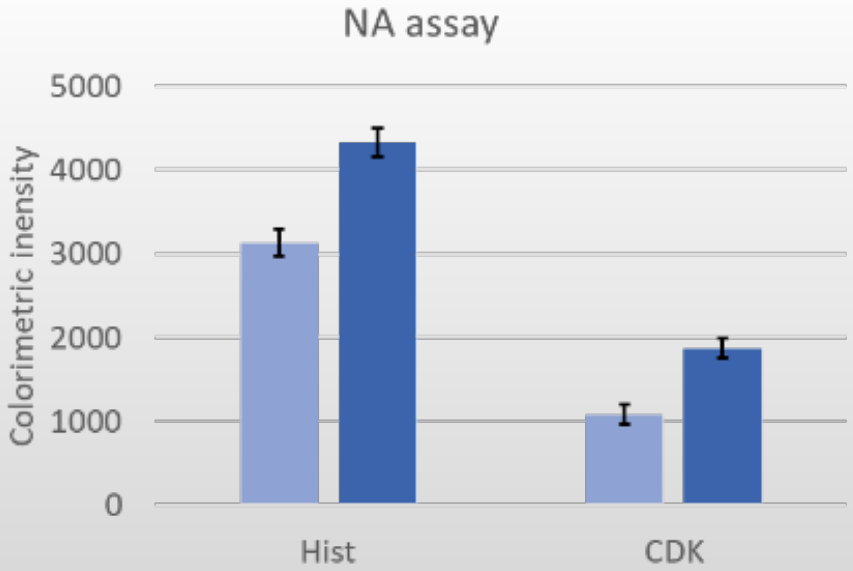
$$t_{res} = \frac{L_s}{u}$$

- Nanopore allows better target capture under high flow speed
- VFI short flow path: 100 μm vs. 40 mm in LFI

…and then adapted for the detection of biodosimetry genes



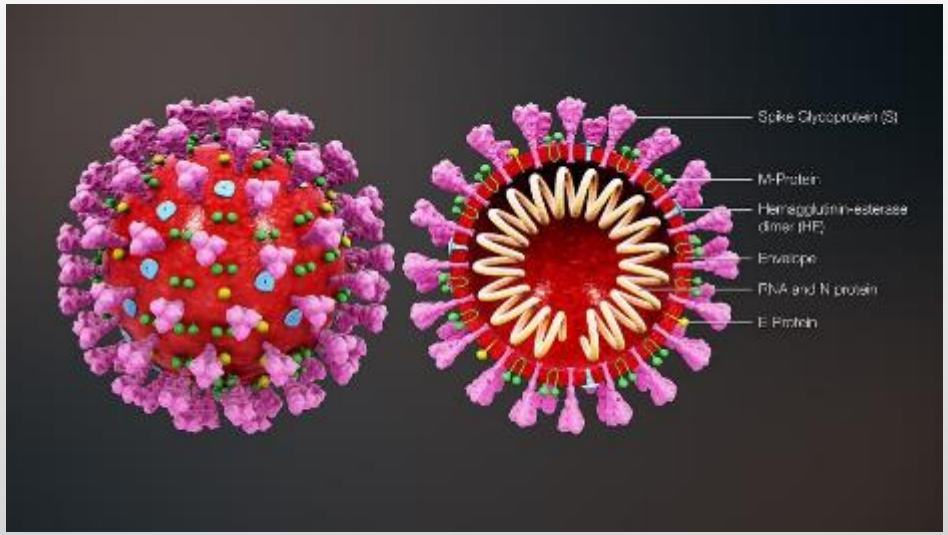




Ratio 5 Gy/0 Gy:

CDKN1A: 1.38
HIST1H3D: 1.9

TRISH# SYN0003 & NNX16A069A

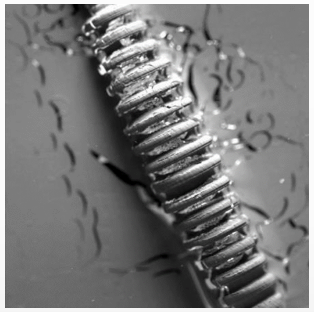

Spike Glycoprotein (S)
M-Protein
Hemagglutinin-esterase dimer (HE)
Envelope
RNA and N protein
E Protein


COVID-19 :

THE UNIVERSITY OF ARIZONA.

CENTER FOR APPLIED NANOBIOSCIENCE & MEDICINE

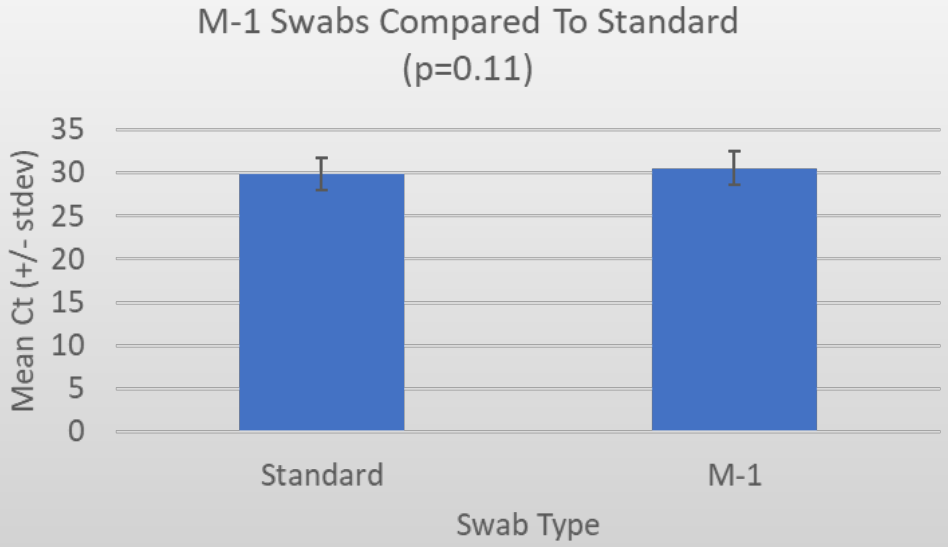
robotics



Many Challenges for Countermeasures:

- Shortage of Supplies
- Sensitive tests
- At home simple test
- Therapeutics
- Vaccine

Saliva PCR test FDA EUA 20OCT20



Recombinant Antibodies: SARS-CoV-2-S protein (Uniprot DB)

ABCD	Ab Name	PMID/DOI	PDB	Epitope	Comments
AI334	CR3022	16796401, 32245784	6W41	S1 (356-504)	Isolated from CoV-1 patient; defined 3D structure
AQ806	VHH-72	32375025	6WAQ	S1 (RBD/355-366)	Nanobody raised against CoV-1; defined 3D structure
AR209-AR271	Sb#XXX (63 Abs)	10.1101/2020.04.16.045419		S1 (RBD/330-526)	Nanobodies (top-performers: AR222, AR223, AR224, AR248, AR249, AR269)
AS273 AS274	B38 H4	32404477	7BZ5	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AS682-AS725	(44 Abs)	32561270	6XE1		Isolated from CoV-2 patient Only 2 neutralizing and targeting RBD (AS682 and AS708); defined 3D structure
AS739	S309	32422645	6WPS	S1 (non-RBD)	Isolated from CoV-1 patient; defined 3D structure
AS740 AS862	CB6 CA1	32454512	7C01	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure; prevents infections in monkeys
AT085	P2B-2F6	32454513	7BWJ	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AT086	NbTy1	10.1101/2020.06.02.130161		S1 (RBD)	Nanobody; defined 3D structure (no PDB)
AT460-AT542 AT961-AT972	(95 Abs)	32555388 10.1101/2020.05.28.121533	N.A.	S1 (RBD)	Isolated from CoV-2 patients; defined 3D structure for AT483
AT693	BD23	32425270	7BYR	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AT798 AT799	W23UACh W25UACh	10.1101/2020.06.09.137935			Nanobodies
AT800-AT828	(29 Abs)	10.1101/2020.06.09.143438	7C8V, 7C8W, 7CAN	S1 (RBD)	Nanobodies; 3 with defined 3D structure (AT801, AT817, AT828)
AT868-AT876	(9 Abs)	32540901	6XDG	S1 (RBD)	Regeneron Abs; REGN10933+REGN10987 (AT870+AT869) in clinical trials
AT877-AT958	(82 Abs)	32540902			Isolated from CoV-2 patients (AT892 and AT916 best neutralizers)
AT959 AT960 AU703-AU733	CC12.1 CC12.3 (+31 Abs)	32540903	6XC2 6XC4	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AT973-AU078	(85 Abs)	10.1101/2020.06.23.165415			Nanobodies; 1 with cryo-EM structure (Sb23/AU015)
AU079-AU113	(35 Abs)	32571838	7C2L		Isolated from CoV-2 patients; only 3 neutralizing (4A8/AU079 S1-NTD, 3H3/AU084 S2, 1D2/AU088 S1-RBD); defined 3D structure for 4A8
AU180	EY6A	32737466	6ZER	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AU182-AU207	(26 Abs)	10.4049/jimmunol.2000583		S1 (RBD)	Raised in mouse against CoV-2, 5 neutralizing (AU197/2B04 with therapeutic potential)
AU260-AU300	(40 Abs)	10.1101/2020.05.22.111005		S1 (RBD)	Isolated from CoV-2 patients; best neutralizers COV2-2130/AU270, COV2-2165/AU271, and COV2-2196/AU299
AU422-AU449	(28 Abs)	32673567		S1 (RBD)	Neutralizing Abs isolated from CoV-2 patients
AU450-AU540	(91 Abs)	32651581		NTD, RBD	Isolated from CoV-2 patients; best neutralizers COV2-2678/AU520 and COV2-2514 (seq not released)
AU605-AU617	(13 Abs)	10.1101/2020.07.24.219857		S1 (RBD)	Nanobodies; best blocking Ab NIH-CoVnb-112/AU616
AU734	Fab 2-4	32698192	6XEY	S1 (RBD)	Isolated from CoV-2 patient; defined 3D structure
AU753	Mab362	32511396		S1 (RBD/ACE2)	Neutralizing Ab, hybridoma raised against CoV-1
AV125	H014	32703908	7CAH	S1 (RBD)	NAb raised against CoV-2; defined 3D structure; with therapeutic potential

The VFI in response to COVID-19 crisis for the detection of both S-protein and N-gene: a proof-of-concept

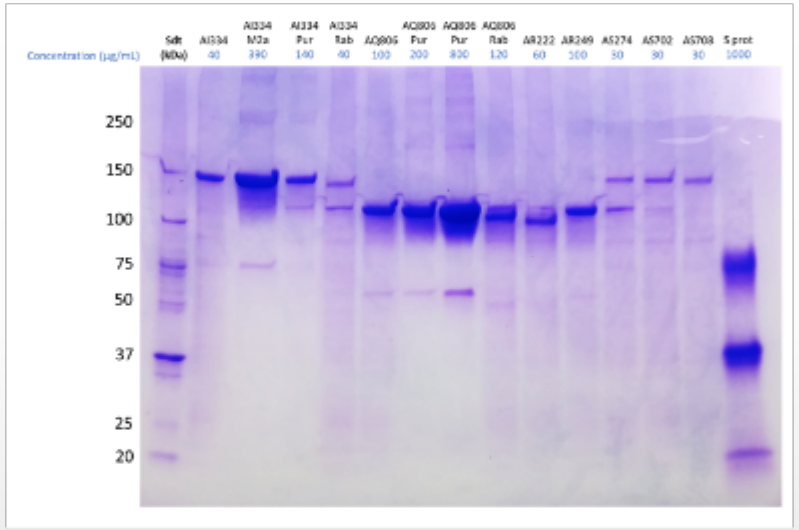
1- Development of anti S-protein antibodies

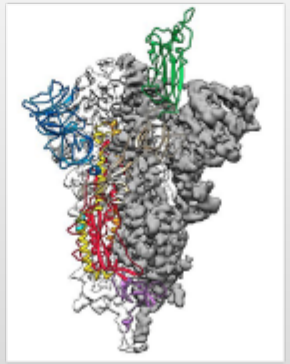
No	Primary Antibodies
1	AI334-mouse
2	AI334-rabbit
3	AQ806-mouse
4	AQ806-rabbit
5	AR222
6	AR249
7	AS274
8	AS702
9	AS708

Previously characterized by direct ELISA:

AI334 & AQ806
Antibody Reports, 2020, vol. 3, e186

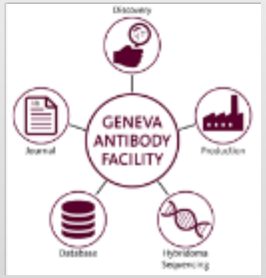
AR222, AR249, AS274, AS702 & AS708
Antibody Reports, 2020, vol. 3, e220





Cryo-EM structure of the 2019-nCoV spike protein
Wrapp et al. Science, 2020

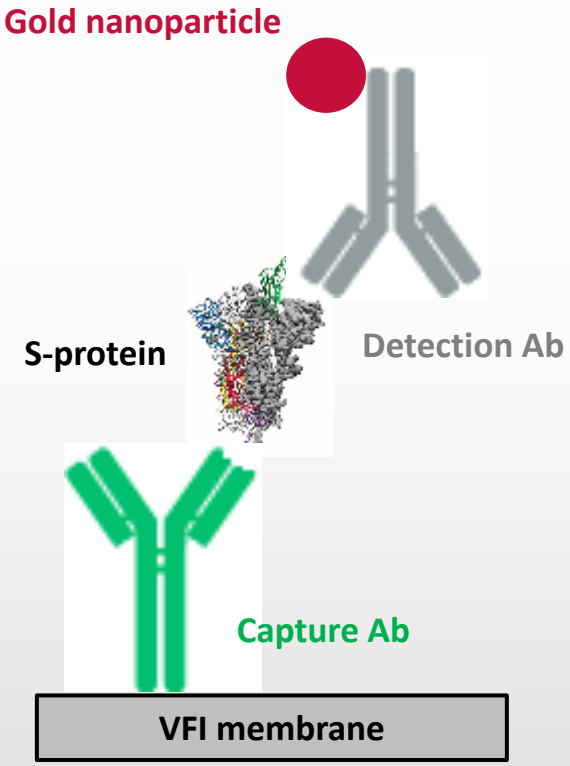
UNIVERSITÉ DE GENÈVE



Development of Sandwich ELISA

Capture Ab	Detection Ab	Signal
AI334-rabbit	AQ806	No signal
AI334-rabbit	AR222	No signal
AI334-rabbit	AR249	No signal
AI334-rabbit	AS702	No signal
AI334-rabbit	AS708	No signal
AI334-rabbit	AS274	No signal
AQ806-rabbit	AI334	No signal
AQ806-rabbit	AR222	Faint
AQ806-rabbit	AR249	Faint
AQ806-rabbit	AS702	Faint
AQ806-rabbit	AS708	Faint
AQ806-rabbit	AS274	Faint
AI334-mouse	AQ806-rabbit	No signal
AR222	AQ806-rabbit	Good
AR249	AQ806-rabbit	Very good
AS702	AQ806-rabbit	Very good
AS708	AQ806-rabbit	Good
AS274	AQ806-rabbit	Good
AQ806-mouse	AI334-rabbit	Very good
AR222	AI334-rabbit	Good
AR249	AI334-rabbit	Very good
AS702	AI334-rabbit	Very good
AS708	AI334-rabbit	Good
AS274	AI334-rabbit	Good

2- Test of different pairs



Development of Sandwich ELISA

Capture Ab	Detection Ab	Signal
AI334-rabbit	AQ806	No signal
AI334-rabbit	AR222	No signal
AI334-rabbit	AR249	No signal
AI334-rabbit	AS702	No signal
AI334-rabbit	AS708	No signal
AI334-rabbit	AS274	No signal
AQ806-rabbit	AI334	No signal
AQ806-rabbit	AR222	Faint
AQ806-rabbit	AR249	Faint
AQ806-rabbit	AS702	Faint
AQ806-rabbit	AS708	Faint
AQ806-rabbit	AS274	Faint
AI334-mouse	AQ806-rabbit	No signal
AR222	AQ806-rabbit	Good
AR249	AQ806-rabbit	Very good
AS702	AQ806-rabbit	Very good
AS708	AQ806-rabbit	Good
AS274	AQ806-rabbit	Good
AQ806-mouse	AI334-rabbit	Very good
AR222	AI334-rabbit	Good
AR249	AI334-rabbit	Very good
AS702	AI334-rabbit	Very good
AS708	AI334-rabbit	Good
AS274	AI334-rabbit	Good

Protocol:

Coat plate with 100 µL/well of **capture Ab at 2 µg/mL**. Incubation overnight at 4oC.
Wash well three times with wash buffer (PBS + 0.5% Tween 20)
Block with 300 µL/well of blocking buffer (PBS + 5% Tween-20 + 0.6% Non-fat dry milk) for 1h at 37°C

Remove blocking buffer and pat dry on paper towel.

Dilute S-protein (1/200 to 1/6400; **30 to 0.94 ng/well**) in dilution buffer (PBS + 5% Tween-20 + 0.6% Non-fat dry milk + 5% NaCl) and add 80 µL/well for 1 h at RT.
Remove samples and wash well three times with wash buffer.

Add 100 µL/well of **detection Ab at 2 µg/mL** for 1 h at RT.
Remove detection Abs and wash well three times with wash buffer

Add 100 µL/well of HRP-conjugated anti-rabbit Ab diluted in dilution buffer (1/5 000) for 1 h at RT.
Remove anti-rabbit Ab and wash well three times with wash buffer.

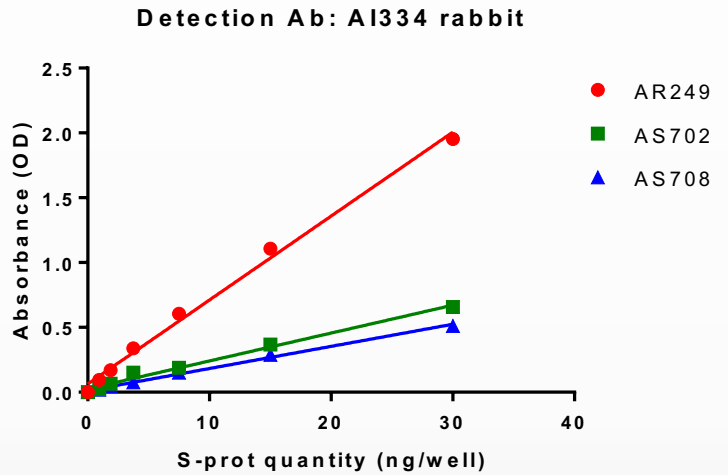
Add 50 µL for ~15 min at RT and add 50 µL of STOP solution
Read plate at 450 nm

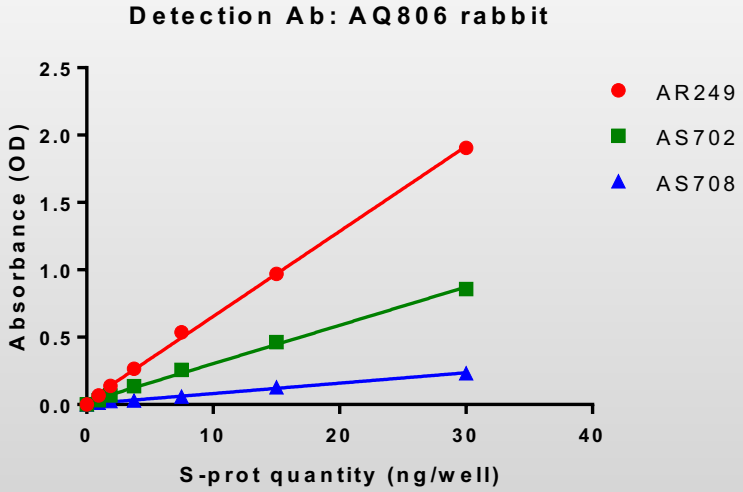
S-protein from BEI resources: Recombinant spike protein (His Tag), SARS-CoV-2 (NR-52308)

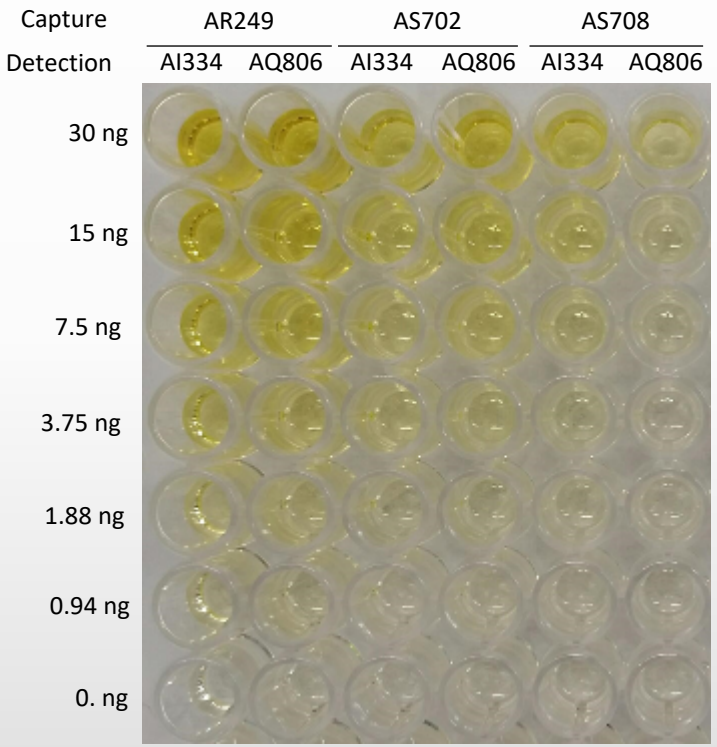
Development of Sandwich ELISA

Capture Ab	Detection Ab	Signal
AI334-rabbit	AQ806	No signal
AI334-rabbit	AR222	No signal
AI334-rabbit	AR249	No signal
AI334-rabbit	AS702	No signal
AI334-rabbit	AS708	No signal
AI334-rabbit	AS274	No signal
AQ806-rabbit	AI334	No signal
AQ806-rabbit	AR222	Faint
AQ806-rabbit	AR249	Faint
AQ806-rabbit	AS702	Faint
AQ806-rabbit	AS708	Faint
AQ806-rabbit	AS274	Faint
AI334-mouse	AQ806-rabbit	No signal
AR222	AQ806-rabbit	Good
AR249	AQ806-rabbit	Very good
AS702	AQ806-rabbit	Very good
AS708	AQ806-rabbit	Good
AS274	AQ806-rabbit	Good
AQ806-mouse	AI334-rabbit	Very good
AR222	AI334-rabbit	Good
AR249	AI334-rabbit	Very good
AS702	AI334-rabbit	Very good
AS708	AI334-rabbit	Good
AS274	AI334-rabbit	Good

3- Dose response curves







VFI integration of sandwich immunoassay

Protocol:

Membrane printed with capture antibody (3nL/spot)

Blocking buffer *(10 mM borate buffer (pH=8) with surfactant (2.5% Triton X-100, 1% BSA, 0.2% PVP-40, 0.1% sucrose))* flowed through the membrane at 0.2 ml/min

In the meantime, incubation for 10 min at RT of S-protein with 5 µL of detection Ab diluted in assay buffer *(0.1 M PB buffer (pH=7.2) with surfactant (0.1% Triton X-100, 0.5% BSA))* and filtration with 0.2 µm PES filter to remove the big particles and aggregated gold nanoparticles

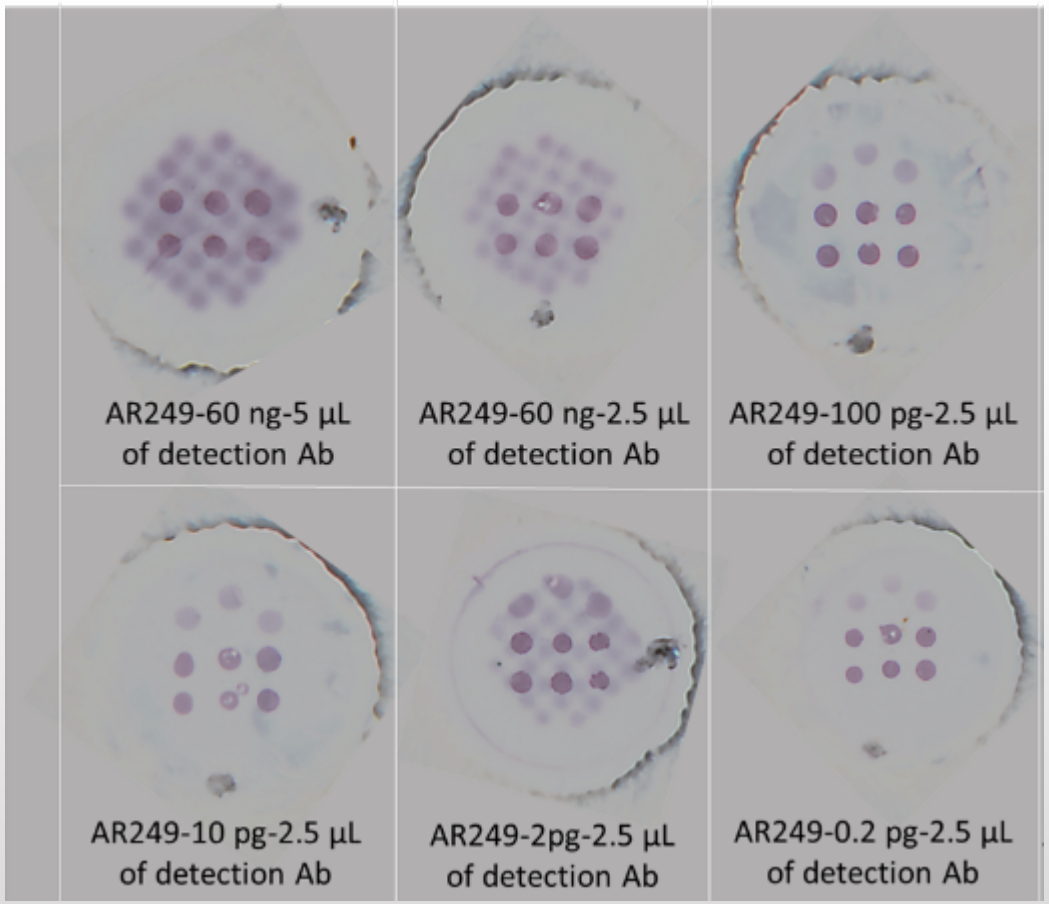
Sample flowed through the membrane at 0.2 ml/min

Assay buffer flowed through the membrane at 0.2 ml/min to wash membrane

Membrane reading

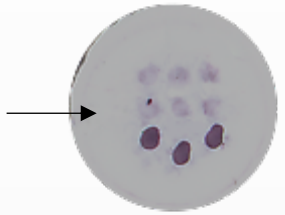
Capture antibody:
High concentrated AR249 (1.6 mg/mL)

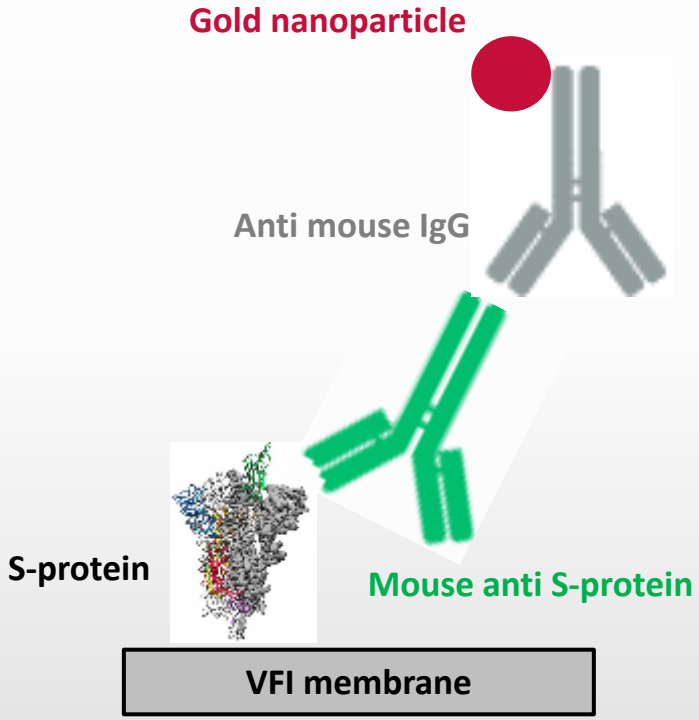
Detection antibody:
AQ806-rabbit (1,78mg/ml) conjugated to gold nanoparticles (OD20)



Indirect ELISA to detect serological response to COVID-19 infection

Capture Ab	Detection Ab	Signal
AI334-mouse	Goat anti-mouse IgG conjugated to gold nanoparticles	Weak signal
AQ806-mouse		Weak signal
AR222		No signal
AR249		Faint signal
AS274		No signal
AS702		No signal
AS708		Weak signal





➡ Weak signal due to low concentration of S-protein

N gene amplification and detection by qPCR

CDC protocol

Target: Spiked plasmid

N gene primers/probe:
Forward (F): GACCCCAAAATCAGCGAAAT
Reverse (R): TCTGGTTACTGCCAGTTGAATCTG
Probe (P):[5HEX]ACCCCGCATTACGTTTGGTGGACC[BHQ1a-Q]

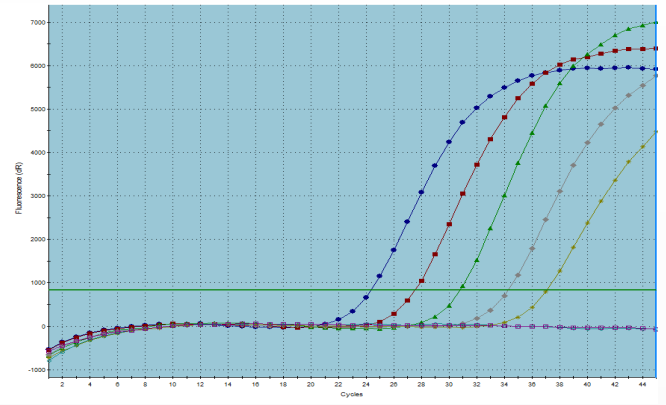
Final concentration: 500 nM (F & R) 125 nM (P)

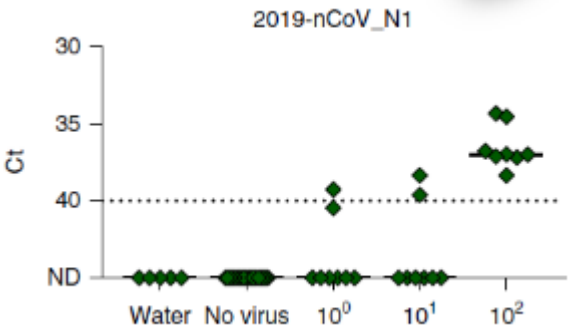
Kit: TaqPath™ **1-Step** Multiplex Master Mix

Thermocycler: Stratagene Mx30005P (Agilent)

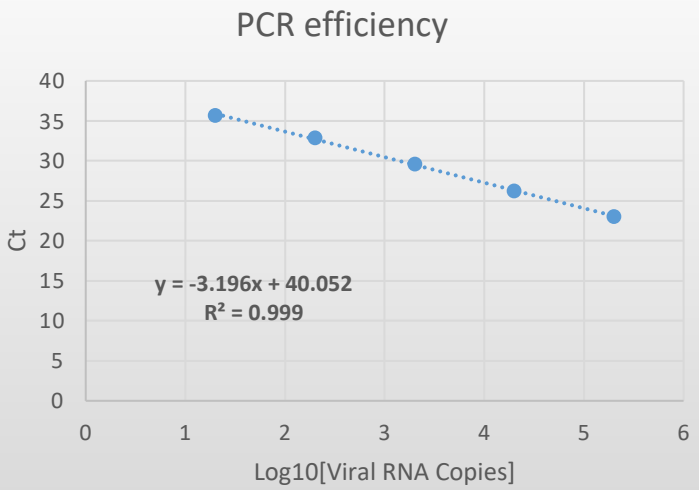
Cycle conditions:

Cycles	Temperature	Time
1	25°C	2 min
1	50°C	10 min
1	95°C	2 min
45	95°C	3 s
	55°C	30 s





Vogels et al. Nat Microbiol. 2020;5(10) 1299-1305



Viral RNA copies/reaction	Ct
200 000	23.05
20 000	26.25
2 000	29.6
200	32.91
20	35.7
2	ND
0	ND

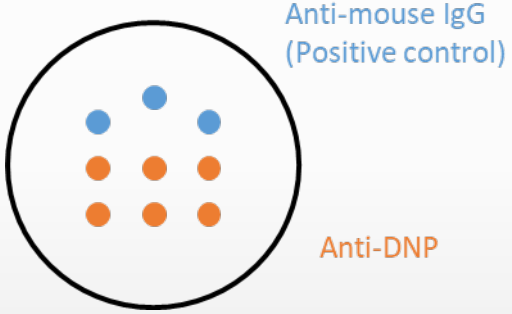
ND = Not Detected

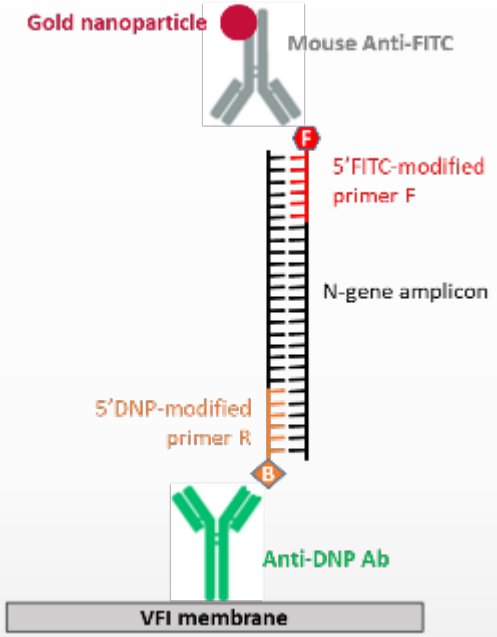
Efficiency = $-1 + 10^{(-1/\text{slope})} \times 100 = 105\%$

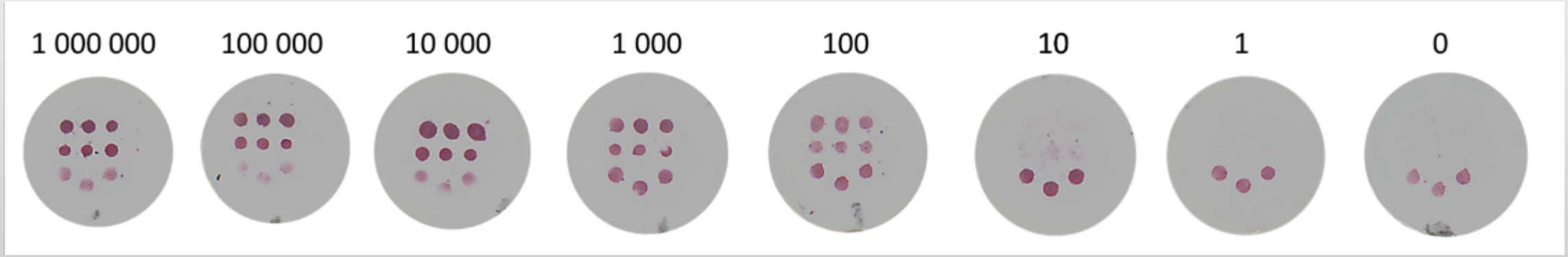
N gene detection with VFI

N gene primers:
Forward (F): [FITC]CACATTGGCACCCGCAATC
Reverse (R): [DNP]GAGGAACGAGAAGAGGCTTG
Corman VM et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25

Kit: 2-steps protocol
RT: RNA to cDNA EcoDry Premix (lyophilized)
PCR: High Yield PCR EcoDry (lyophilized)







XPRIZE nasal and saliva samples results for COVID-SARS2 detection: VERIFAST vs PCR

157 blinded samples with different matrixes (saliva, nasal swabs, PBS, water) tested in 2 days

Sample type	VERIFAST result	PCR result	Sample type	VERIFAST result	PCR result
Nasal	+	+	Nasal	-	-
Nasal	+	+	Saliva	+	-
Saliva	+	-	Nasal	+	+
Saliva	+	-	Nasal	failed	+
Nasal	+	+	Saliva	+	+
Saliva	+	+	Nasal	+	+
Nasal	+	+	Nasal	+	+
Saliva	+	+	Saliva	+	+
Nasal	+	+	Saliva	failed	+
Nasal	+	+	Nasal	+	+
Saliva	+	+	Saliva	+	+
Saliva	+	+	Nasal	+	+
Nasal	+	+	Nasal	+	+
Saliva	+	+	Nasal	+	+
Nasal	-	-	Nasal	+	+
Saliva	+	+	Saliva	+	+
Nasal	+	+	Saliva	failed	+
Nasal	+	+	Saliva	+	+
Saliva	+	-	Saliva	+	+
Saliva	+	+			

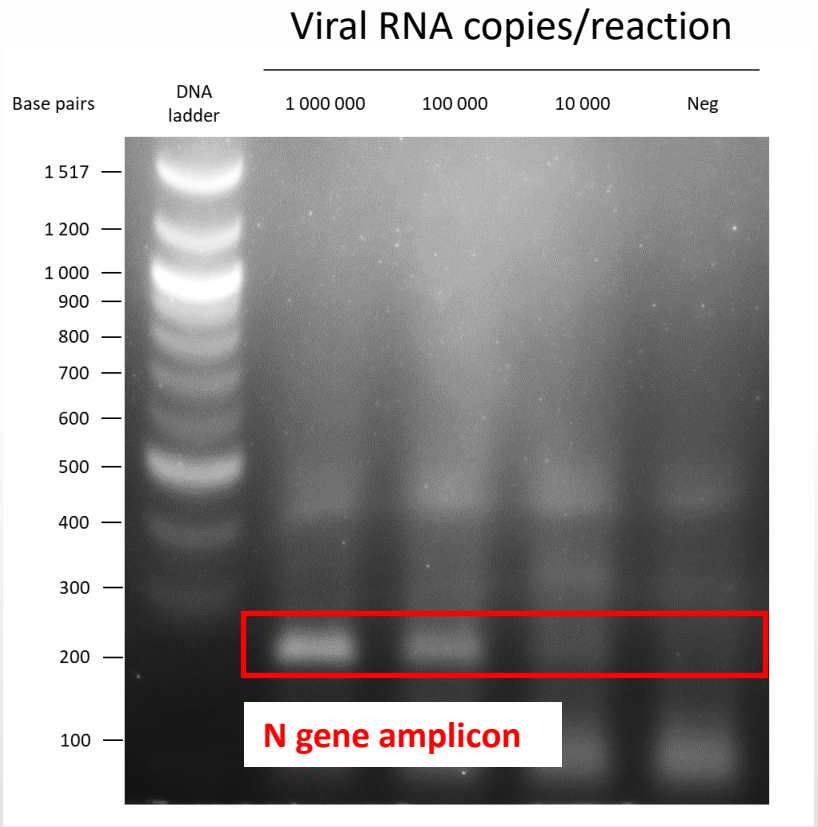
Development of one-step isothermal Amplification

TwistAmp Basic (TwistDx) protocol

N gene primers:
Forward (F): [DNP]CTAATCAGACAAGGAACTGATTACAAACATTG
Reverse (R): [FITC]CTTATTCAGCAAAATGACTTGATCTTTGAA

Incubation for 20 min at 42°C

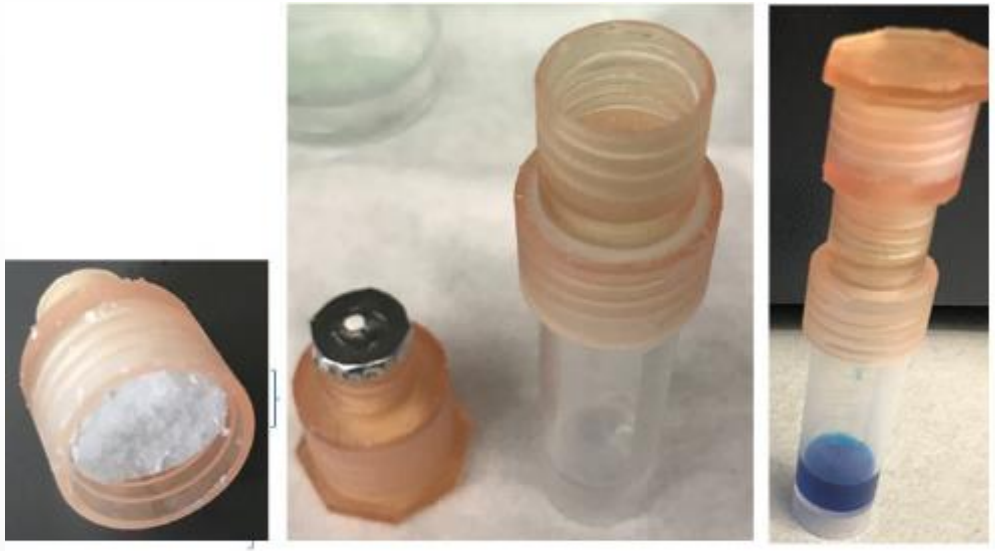
(1-step protocol: RT + RPA)

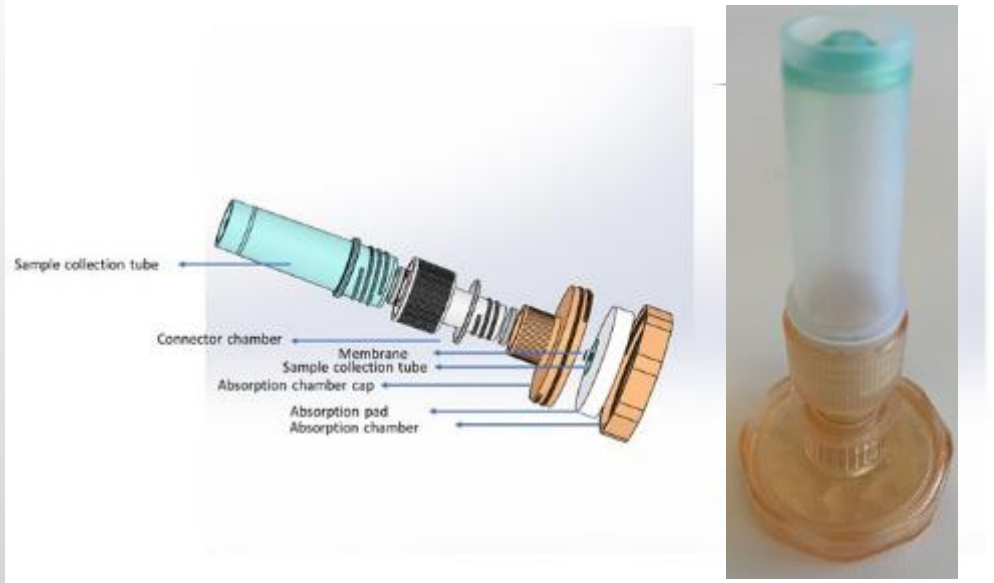


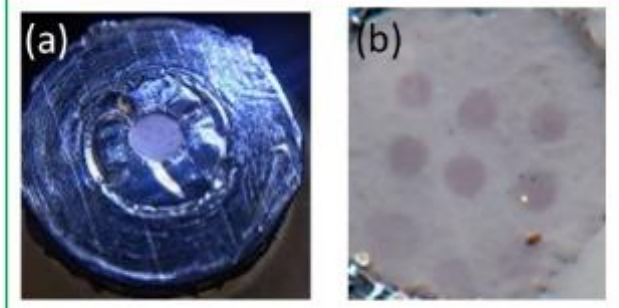
Next steps :

- Test on VFI: sensitivity/Std qPCR and VFI, dose-dependent response, …

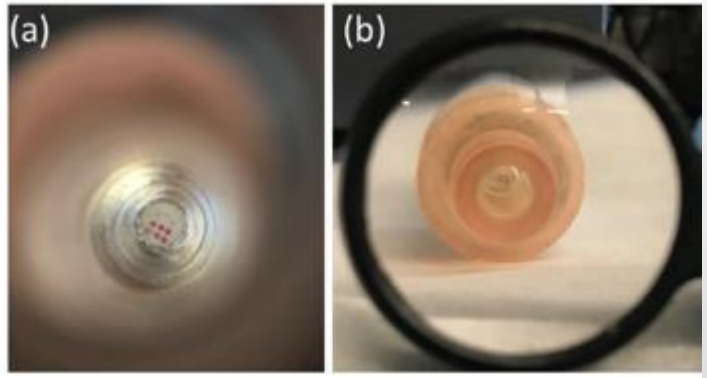
Development of CAP-VFI





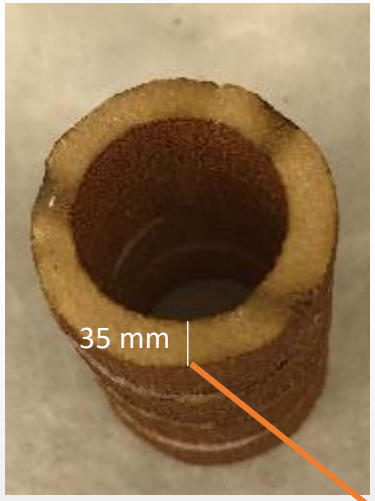


Covid-19 detection (AR249 antibody): (a) image captured by cellphone, (b) scanned by scanner



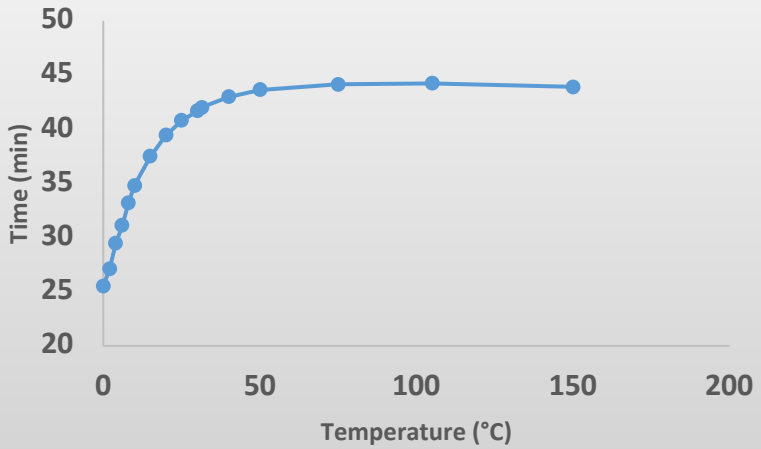
Detection of COVID-SARS2 (200 copies of N-gene) using simple VFI: (a) image captured by lens adaptor, (b) image captured using a magnifier

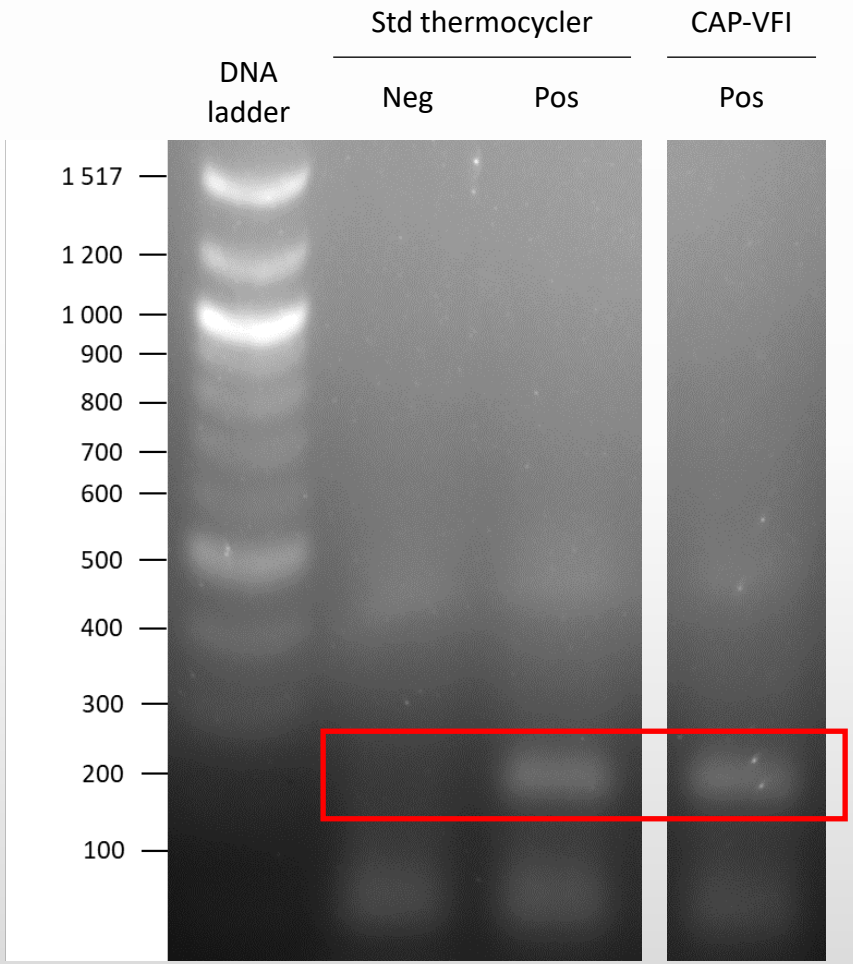
Integration of RT+RPA in CAP-VFI



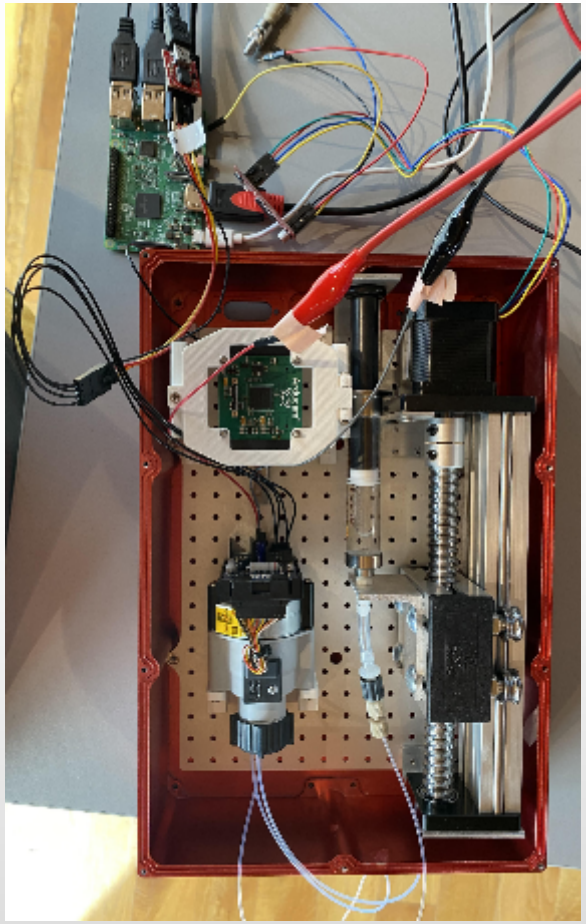
Isolation layer

After 40 min, temperature reach to 43 °C





Back to the Future: Automated System for Space

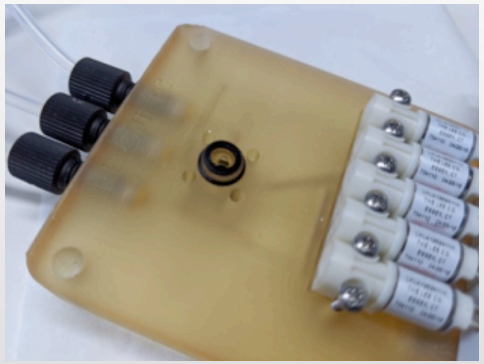


Automated VFP system in 6U CubeLab

a

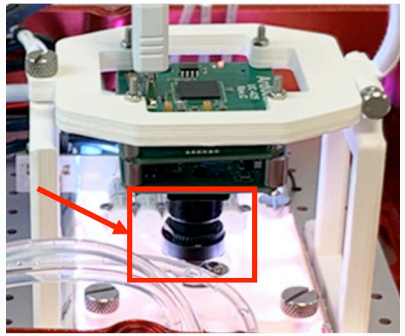
Automated VFP prototype in CubeLab

b



Membrane Holder and valves

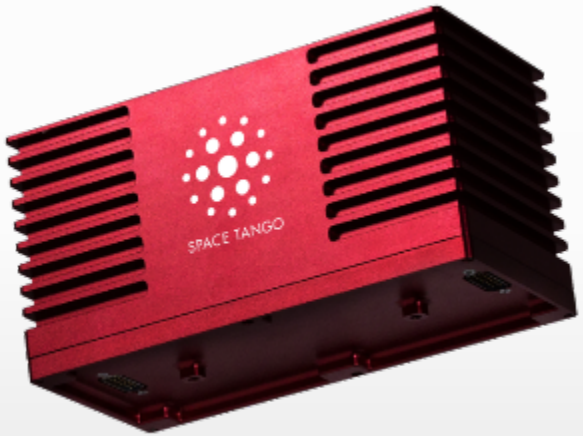
c



Camera for Imaging

d

Captured Image



Automated VFP system

Conclusion

- VFI platform is versatile for integrating several molecular assay tests from biofluids
- Demonstration of potential in COVID-19 to detect simultaneously viral particles (viral protein and/or (different) genes) + immune response (antibodies)
- Next configuration with new saliva collection kit will provide a full at home solution
- Validation for next FDA EUA and production
- Exploration for support from federal agencies and industry

Thank you