Translating scientific challenges into preclinical research solutions

World Vaccine Conference, Oct. 18, 2023

Konrad Stadler

Cerba Research and Viroclinics-DDL

- A Global Contract Research Organization with more than 50 years of Specialty Diagnostic and Laboratory Services.

- Specialty Services in Virology & Immunology through its specialty BU Viroclinics-DDL.

- One Stop Shop combining Routine and Specialty testing.

- Ranging from pre-clinical testing to support of Phase III studies and Global Logistics.

Our Global Footprint

A Network of Excellence

- Research & Partner labs
- Pre-Processing labs
- Office locations and Technical Platforms

USA:

New York
Routine, Genetics/NGS, Molecular Biology, IVD, Flow Cytometry, Bioanalytical Lab/PK, Histopathology/IHC, Virology

China:

Shanghai
Routine, Genetics/NGS, Molecular Biology, Flow Cytometry, Bioanalytical Lab/PK, Histopathology/IHC

France:

Paris & Montpellier
Genetics/NGS, Molecular Biology, IVD, Flow Cytometry, Histopathology/IHC, Virology, Microbiology, Pre-clinical, Biorepository

Taiwan:

Taipei
Routine, Genetics/NGS, Flow Cytometry, Histopathology/IHC Lab/PK,, Histopathology/IHC

South Africa:

Johannesburg, Durban, Cape Town
Routine, Genetics/NGS, Molecular Biology, Flow Cytometry,Histopathology/IHC, Virology, Microbiology, BSL3, Biorepository, PBMC

Australia:

Sydney
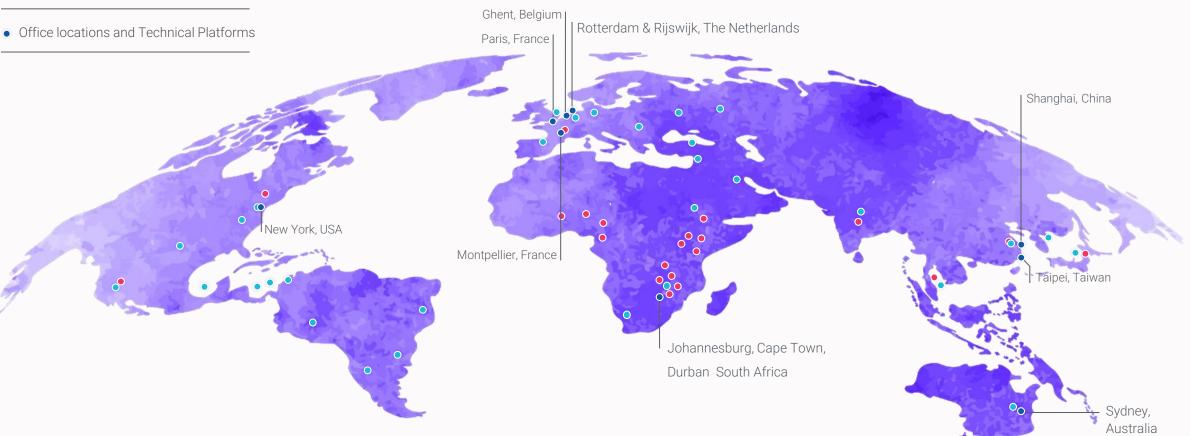Routine, Molecular Biology, Flow Cytometry, Virology

Belgium:

Ghent
Routine, Molecular Biology, Bioanalytical Lab, Flow Cytometry, Microbiology
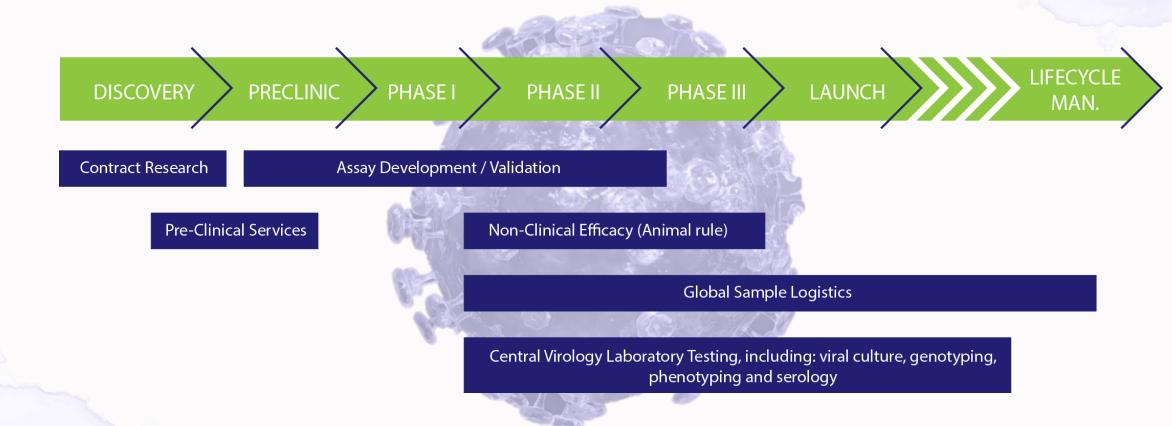
The Netherlands:

Rotterdam, Rijswijk & Schaijk
BSL3, Pre-clinical, Molecular Biology, Genetics/NGS, Pathology, Virology

Ghent, Belgium

Rotterdam & Rijswijk, The Netherlands

Paris, France

Shanghai, China

New York, USA

Montpellier, France

Taipei, Taiwan

Johannesburg, Cape Town,

Durban South Africa

Sydney, Australia

Viroclinics - a one-stop shop for vaccine research

DISCOVERY → PRECLINIC → PHASE I → PHASE II → PHASE III → LAUNCH → LIFECYCLE MAN.

Contract Research

Assay Development / Validation

Pre-Clinical Services

Non-Clinical Efficacy (Animal rule)

Global Sample Logistics

Central Virology Laboratory Testing, including: viral culture, genotyping, phenotyping and serology

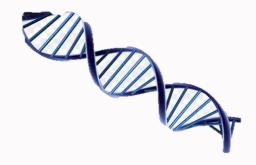Detection and molecular characterization of (emerging) viruses

Animal models

Laboratory assays for virus testing and evaluation of virus-specific immune responses

Viral sequencing applications – long & short read

Sequencing Applications

- ❑ Direct RNA sequencing
- ❑ Monitor quasispecies or the emergence of virus variants (combination of short and long read sequencing)
- ❑ Understand the spread of a novel virus or virus mutants through communities
- ❑ Identify conserved regions and mutational hotspots within the genome
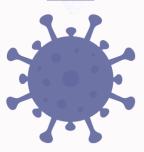- ❑ B-, T-cell receptor repertoires
- ❑ … (more)

Technology

Virus

- Sars-Cov-2
- hRSV
- hMPV
- Influenza
- Dengue
- HBV/HDV/HCV
- HPV
- HIV
- CMV
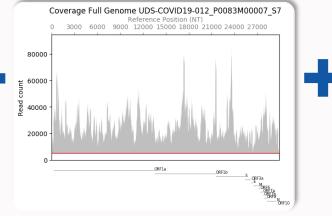- And more ….

Dedicated Bio-IT Team & proprietary pipeline

BIG DATA

INFORMATION

FASTQ files

Reference seq

```
>Wuhan-Hu-1
ATTAAAGGTTTA
TACCTTCCCAGG
TACAAACCA...
```

Variant table

```
A100T    @ 10%
C105T    @ 2%
G137A    @ 87%
C223DEL  @ 43%
...
```

- Substitutions (A23403G)
- Insertions (21990.1T)
- Deletions (A21766DEL)

Coverage graph (# nt per genomic position)

Coverage Full Genome UDS-COVID19-012_P0083M00007_S7

Consensus seq

```
>Sample14
ATTACCGGTTT
ATACCTTCCCA
GGTACAATCCA
...
```

Joint publications FDA

Detection and molecular characterization of new viruses

Animal models

Laboratory assays for virus testing and evaluation of virus-specific immune responses

Capabilities and preclinical expertise

- 1600 m^2 preclinical facility in Schaijk, the Netherlands

- Conventional, BSL2 and BSL3(+) animal facility

- GAP III Polio Certificate of Participation

- Negatively-pressurized isolator cages

- Range of different animal species

- Solutions for any kind of experimental set up: challenge and transmission studies

- AAALAC-accreditation (In progress)

Research Types

- Vaccine Efficacy
- Passive Immunization
- Immunogenicity
- Transmission studies
- Antiviral Therapy/Prophylaxis
- Immunopathology
- Pathogenicity
- Pharmaco Kinetics
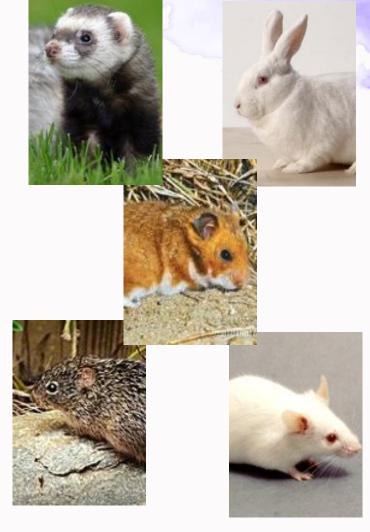- Resistance / Mutants
- Safety
- ...(*more*)

Lab Read Outs

- Virus titer
- Virus genome copy number
- Antibody
- Cytokines
- Pathology
- Histopathology
- Genomics
- Imaging
- ... (*more*)

Development of animal models for any pathogen and research question

Virus	Mouse	Ferret *	NHP	Other
H7N9 A/Influenza	x	X		
H5N1 A/Influenza	X	X	X	Cat
pH1N1 A/Influenza	X	X**	X	Cat
H3N2 A/Influenza	X	X **		
B/Influenza	X	X		
HRSV (A and B)	X	X **	X	Cotton rat
hMPV	X		X	Hamster
SARS-CoV		X		Cat
MERS-CoV				Rabbit
SARS-CoV-2		X		Hamster, rabbits
Varizella Zoster virus			X	
Measles virus			X	Cotton rat
Monkeypox virus			X	
Rabies virus	X			
Dengue virus			X	
Polioviruses	X			
… (more)				

*   Naive and pre-exposed ferrets
** Immunocompromised ferret model

SARS-CoV-2 Model Development Timelines

Dec 2019
First case of SARS-CoV-2 in humans reported

Feb 2020
Virus stock available for animal model development

Mar/Apr 2020
Syrian Golden Hamster established as SARS-CoV2 animal model

Animal models for COVID-19

César Muñoz-Fontela, William E. Dowling, Simon G. P. Funnell, Pierre-S. Gsell, A. Ximena Riveros-Balta, Randy A. Albrecht, Hanne Andersen, Ralph S. Baric, Miles W. Carroll, Marco Cavaleri, Chuan Qin, Ian Crozier, Kai Dallmeier, Leon de Waal, Emmie de Wit, Leen Delang, Erik Dohm, W. Paul Duprex, Darryl Falzarano, Courtney L. Finch, Matthew B. Frieman, Barney S. Graham, Lisa E. Gralinski, Kate Guilfoyle, Bart L. Haagmans, Geraldine A. Hamilton, Amy L. Hartman, Sander Herfst, Suzanne J. F. Kaptein, William B. Klimstra, Ivana Knezevic, Philip R. Krause, Jens H. Kuhn, Roger Le Grand, Mark G. Lewis, Wen-Chun Liu, Pauline Maisonnasse, Anita K. McElroy, Vincent Munster, Nadia Oreshkova, Angela L. Rasmussen, Joana Rocha-Pereira, Barry Rockx, Estefanía Rodríguez, Thomas F. Rogers, Francisco J. Salguero, Michael Schotsaert, Koert J. Stittelaar, Hendrik Jan Thibaut, Chien-Te Tseng, Júlia Vergara-Alert, Martin Beer, Trevor Brasel, Jasper F. W. Chan, Adolfo García-Sastre, Johan Neyts, Stanley Perlman, Douglas S. Reed, Juergen A. Richt, Chad J. Roy, Joaquim Segalés, Seshadri S. Vasan, Ana María Henao-Restrepo ✉ & Dan H. Barouch ✉
- Show fewer authors

Nature **586**, 509–515(2020) | Cite this article

Apr/May 2020
First efficacy testing of a SARS-CoV2 vaccine candidate in hamsters

Ad26.COV2.S-elicited immunity protects against G614 spike variant SARS-CoV-2 infection in Syrian hamsters and does not enhance respiratory disease in challenged animals with breakthrough infection after sub-optimal vaccine dosing

Authors

Joan E.M. van der Lubbe[1]*, Sietske K. Rosendahl Huber[1], Aneesh Vijayan[1], Liesbeth Dekking[1], Ella van Huizen[1], Jessica Vreugdenhil[1], Ying Choi[1], Miranda R.M. Baert[1], Karin Feddes-de Boer[1], Ana Izquierdo Gil[1], Marjolein van Heerden[2], Tim J. Dalebout[3], Sebenzile K. Myeni[3], Marjolein Kikkert[3], Eric J. Snijder[3], Leon de Waal[4], Koert J. Stittelaar[2], Jeroen T.B.M. Tolboom[1], Jan Serroyen[1], Leacky Muchene[1], Leslie van der Fits[1], Lucy Rutten[1], Johannes P.M. Langedijk[1], Dan H. Barouch[6], Hanneke Schuitemaker[1], Roland C. Zahn[1], Frank Wegmann[1]
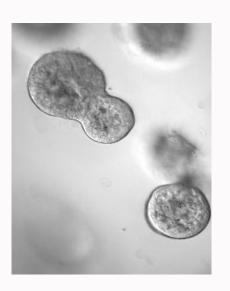
Npj Vaccines (2021)6:39

Organoids - Complement to animal models

- [ ] Human system
- [ ] Derive from a few cells from a tissue, embryonic stem cells or induced pluripotent stem cells
- [ ] Minituarized version of organs or tissues produced in vitro → recapitulate morphology and functions of their in vivo counterparts
- [ ] Study pathogen-host interaction in vitro
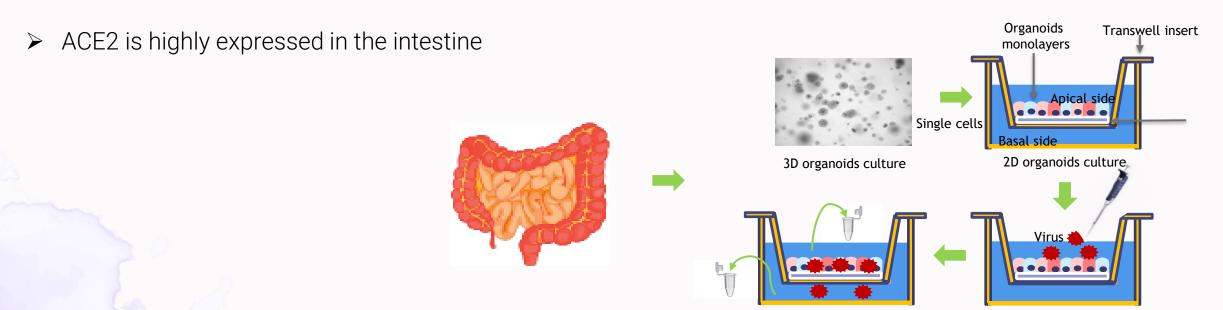- [ ] Model system for drug screening, discovery and development

Human gut organoids to study SARS-CoV2 infection of the gastrointestinal tract

➢ COVID-19 patients experience gastrointestinal symptoms (e.g. diarrhea).

➢ SARS-CoV2 can be detected in stool samples (infectious particles)

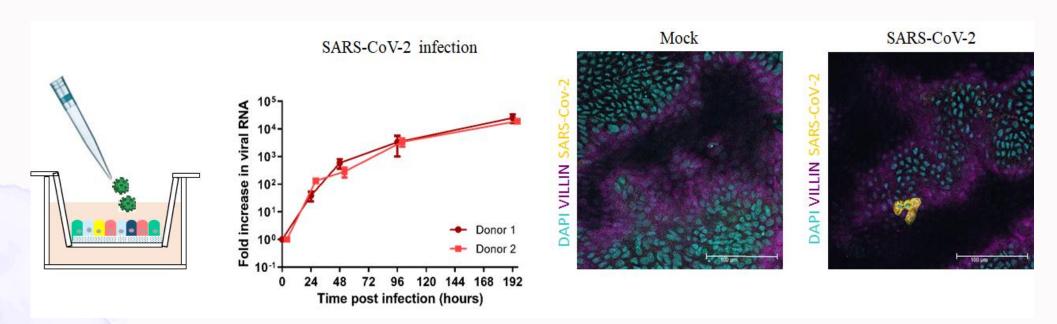➢ SARS-CoV2 spread via so-called 'fecal-oral transmission'.

➢ ACE2 is highly expressed in the intestine

3D organoids culture

Organoids monolayers

Transwell insert

Apical side

Basal side

Single cells

2D organoids culture

Virus

Organoids(3D) were dissociated by enzymatic treatment into single cells and cultured on transwells (2D) to facilitate virus infection

SARS-CoV2 replicates in human intestinal epithelium and infects enterocytes

❏ Cells infected apically with SARS-CoV2

❏ Two different donors used

❏ Apical side sampled 0, 24, 48, 96 and

192 hours post infection

Ikrame Aknouch et al.; Life Sci Alliance. 2022 Dec; 5(12)

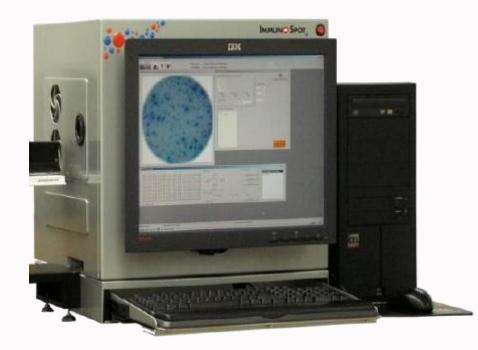Detection and molecular characterization of new viruses

Animal models

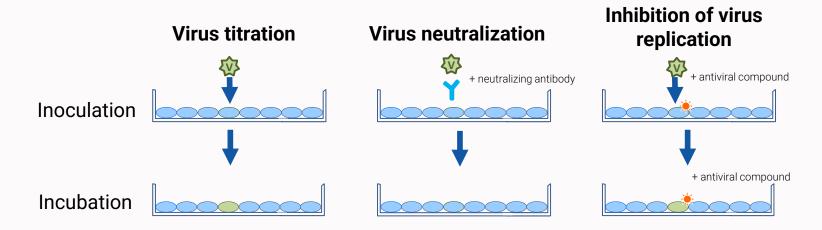Laboratory assays for virus testing and evaluation of virus-specific immune responses

ViroSpot assay – a versatile platform for high-throughput virology testing

❑ Used for:
- Infectious virus titration
- Virus neutralization and inhibition
- Antigenic characterization of virus strains
- Monitoring virus phenotype associated with different susceptibility

❑ The assay is performed with wildtype virus → gold standard for evaluation of functional antibodies

❑ CTL ImmunoSpot analyser
resolution of immuno-staining is sufficient to detect and count single infected cells (GLP and 21CFR part 11 compliant software)
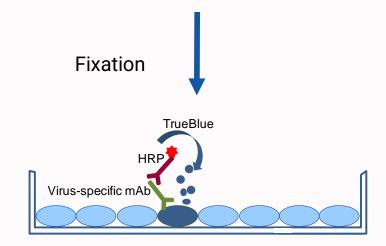
ViroSpot to measure virus infectivity, neutralization and inhibition of virus replication

Virus titration

Virus neutralization

Inhibition of virus replication

+ neutralizing antibody

+ antiviral compound

Inoculation

Incubation

+ antiviral compound

Incubation period depends on virus replication kinetics and on aim of the assay
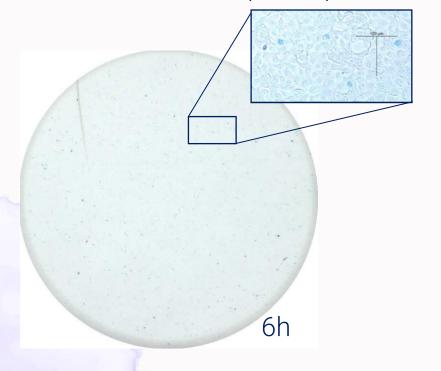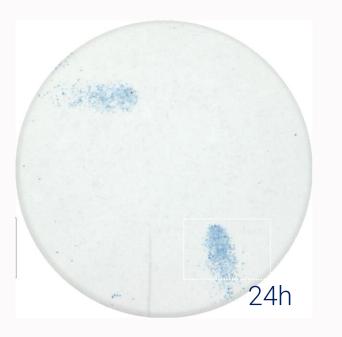
Fixation

TrueBlue

HRP

Virus-specific mAb

Immunostain ViroSpots

ViroSpot assay: Influenza A virus-infected MDCK cells

- A(H3N2) strain A/Perth/16/2009

- 6h: Infected cells detectable

- 24h: comet shaped infected area in absence of solidifying agent (e.g. CMC)
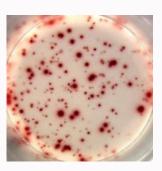
  well defined spots in presence of CMC

6h

24h

+ Carboxymethyl cellulose (CMC)

Other assays for evaluation of immune responses in pre-clinical setting

○ ELISpot/FluoroSpot

• The ELISpot assay is one of the most sensitive immunoassay for *ex vivo* enumeration of antigen-specific B and T cells secreting signature molecules (e.g. cytokines and antibodies) at the single cell level.

○ ELISA

○ MesoScaleDiagnostics (MSD) platform

○ Luminex

○ Cytotoxicity assays (LDH release, WST-8, Cell Titer Glow)

Cytokine secreting cells
(T cells)

Antibody secreting cells
(B cells)

Summary

❑ Viroclinics one-stop shop for vaccine research

❑ Extensive experience and know-how in research on endemic and (re)emerging pathogens

❑ Our research and preclinical activities encompass molecular characterization of viruses, development of animal models, and laboratory assays needed for vaccine development.

❑ Viroclinics/Cerba Research is partner of the Coalition for Pandemic Preparedness Innovation (CEPI) centralized laboratory network

For more information visit our booth N. 60