

Cerba Research the Netherlands

Leon de Waal, PhD

Principal Scientist Preclinical Services



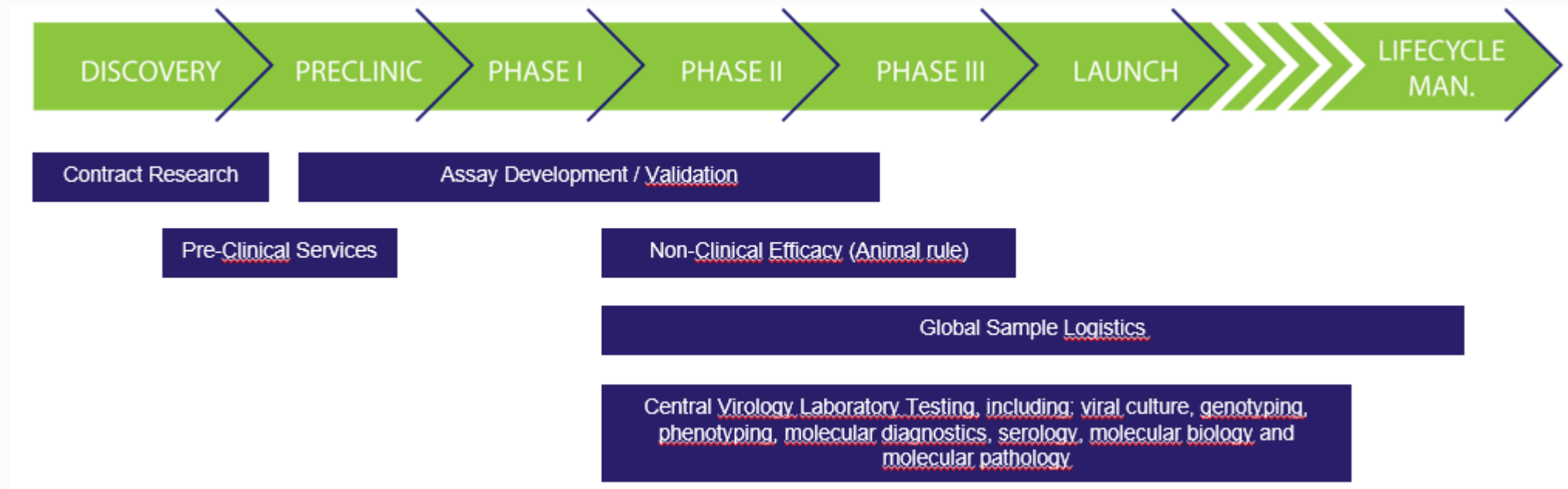
Viroclinics-DDL

A Cerba Research Company

Cerba Research



- 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 Cerba Research the Netherlands.
- One Stop Shop combining Routine and Specialty testing for intervention strategies against wide range of targets.
- Ranging from exploratory/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

France:

Paris & Montpellier

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

South Africa:

Johannesburg, Durban, Cape Town

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

Belgium:

Ghent

Routine, Molecular Biology, Bioanalytical Lab, Flow Cytometry, Microbiology

China:

Shanghai

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

Taiwan:

Taipei

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

Australia:

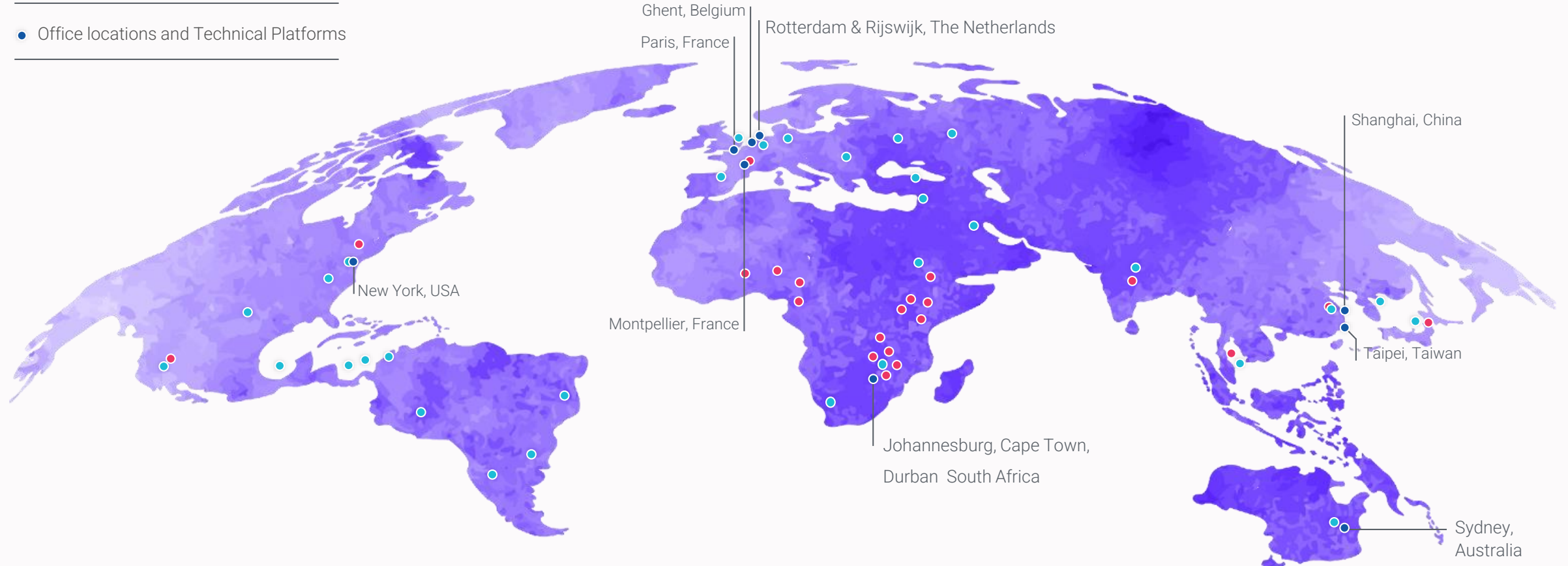
Sydney

Routine, Molecular Biology, Flow Cytometry, Virology

The Netherlands:

Rotterdam, Rijswijk & Schaijk

BSL3, Pre-clinical, Molecular Biology, Genetics/NGS, Pathology, Virology





Outline of the presentation

Preparedness in general

Preclinical Animal Models

Clinical sample testing: one stop shop

Global Logistics

Lessons Learned for next Pandemic

Preparedness necessities (endemic & pandemic)

Models

- Cell culture systems:
 - Internal cell bank
 - Commercially available cell banks
 - Ex vivo materials
 - Specialised cell cultures / Organoids
- Preclinical models
 - Next section

Viruses

Internal Collection	Commercially Available	Non/Semi-Commercially Available	Academic Collaborations	Other Sources
Past and current strains Biorepository	ATCC BEI Resources ECACC	NIBSC EVAg	Erasmus MC University of Hannover	Wildlife monitoring Institutes Animal Health Institutes

Models

- Cell culture systems:
 - Internal cell bank
 - Commercially available cell banks
 - Ex vivo materials
 - Organoids
- Preclinical models
 - Next section

- **Circulating clinically relevant endemic virus strains**
- **Recent pandemic virus strains**

Viruses



Models

- Cell culture systems:
 - Internal cell bank
 - Commercially available cell banks
 - Ex vivo materials
 - Organoids
- Preclinical models
 - Next section

- **Circulating clinically relevant endemic virus strains**
- **Recent pandemic virus strains**

Viruses

Availability of H5N1 clade 2.3.4.4b

- **Mamalian origin (seal, TiHo)**
- **Avian origin (chicken, Royal GD)**



Preclinical Model Development and Intervention studies

Preclinical Expertise and Capabilities

- Preclinical facility for SPF, Conventional, BSL2 and BSL3(+)
- Wide Range of different preclinical animal models (wild-type and GMO)
- Solutions for any kind of experimental set up
- Polio High Containment Facility (GAPIV) for clinical sample testing
 - => Serological testing (Sabin and Salk)
 - => mTgmNVT (nOPV programs)

Viral Targets

Influenza (seasonal and pandemic potential/zoonotic)
Common cold viruses (RSV, hMPV)
Beta corona viruses (MERS-CoV, SARS1, SARS-CoV-2)
Non-respiratory viruses (Polio, Rabies, Dengue)
...and more

Models

Mice (wt and Tg)
Ferrets
Rabbits
Hamsters
Cotton Rats
....and more

Type of research

Direct infection and transmission
Immunogenicity/vaccine efficacy
PK/antiviral efficacy
GLP
...and more

Read Outs

Virus Titers

Virus Copies

Antibody

CMI

Genomics

Pathology

Histopathology

Mortality

Clinical Signs

Imaging

...(more)



Preclinical Model Development

Choice of Model (Species)
Alternatives?

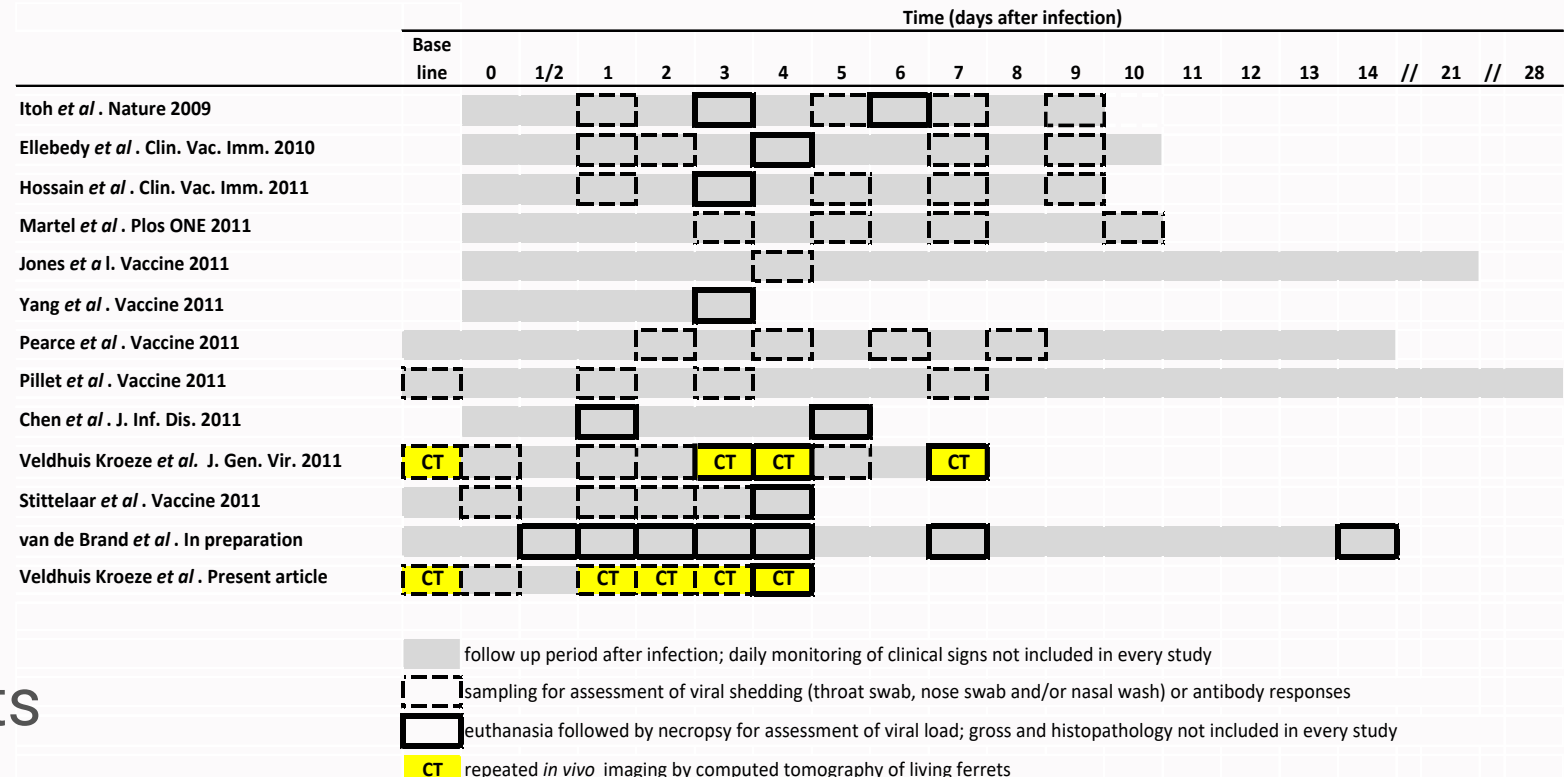
Choice of Pathogen

Inoculation Dose

Inoculation Route

Diagnostic/Clinical Read-Outs

Sampling & End Points



Matching Each Pathogen to the right Animal Model



SARS1 & Influenza



MERS-CoV



SARS-CoV-2 (VoC)



RSV



Polio – PVR-Tg mice



Preclinical Efficacy testing

Pathogens

Seasonal influenza virus

Highly pathogenic influenza virus

HRSV

Rabies

MERS-CoV

SARS-CoV

SARS-CoV-2

.....

Seasonal Influenza Vaccine Provides Priming For A/H1N1 Immunization

Giuseppe Del Giudice,¹ Koert J. Stittelaar,² Geert van Amerongen,² James Simon,² Albert D. M. E. Osterhaus,^{2,3} Klaus Stöhr,¹ Rino Rappuoli^{1*}

(Published 23 December 2009; Volume 1 Issue 12 12re1)

Longevity of the protective immune response induced after vaccination with one or two doses of AS03_A-adjuvanted split H5N1 vaccine in ferrets

Benoît Baras^{a,*}, Koert J. Stittelaar^b, Thijs Kuiken^c, Valérie Jacob^a, Roger Bernhard^d, Sandra Giannini^a, Leon de Waal^b, Geert van Amerongen^b, James H. Simon^b, Albert D.M.E. Osterhaus^{b,c}, Emmanuel Hanon^a, Sally P. Mossman^a

Highly potent anti-SARS-CoV-2 multi-DARPin therapeutic candidates

Marcel Walser^{1,*}, Sylvia Rothenberger^{2,3,*}, Daniel L. Hurdiss^{4,5,*}, Anja Schlegel¹, Valérie Calabro¹, Simon Fontaine¹, Denis Villemagne¹, Maria Paladino¹, Tanja Hospodarsch¹, Alexandra Neculcea², Andreas Cornelius¹, Patricia Schildknecht¹, Mirela Matzner¹, Martin Hänggi¹, Marco Franchini², Yvonne Kaufmann¹, Doris Schaible¹, Iris Schlegel¹, Chloe Iss¹, Thamar Looser¹, Susanne Mangold¹, Christel Herzog¹, Dieter Schiegg¹, Christian Reichen¹, Filip Radom¹, Andreas Bosshart¹, Andreas Lehmann¹, Micha A. Haeuptle¹, Alexander Zürcher¹, Toni Vagt¹, Gabriel Sigrist¹, Marcel Straumann¹, Karl Proba², Niina Veitonmäki¹, Keith M. Dawson¹, Christof Zitt¹, Jennifer Mayor^{2,3}, Sarah Ryter², Heyrhyoung Lyoo⁴, Chunyan Wang⁴, Wentao Li⁴, Ieva Drulyte⁶, Wenjuan Du⁴, H. Kaspar Binz⁷, Leon de Waal⁸, Koert J. Stittelaar^{8*}, Sarah Taplin⁹, Seth Lewis¹, Daniel Steiner¹, Frank J.M. van Kuppeveld⁴, Olivier Engler², Berend-Jan Bosch⁴, Michael T. Stump^{1,10}, Patrick Amstutz¹

mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents

Susanne Rauch^{*1}, Nicole Roth¹, Kim Schwendt¹, Mariola Fotin-Mleczek¹, Stefan O. Mueller¹, Benjamin Petsch^{*1}

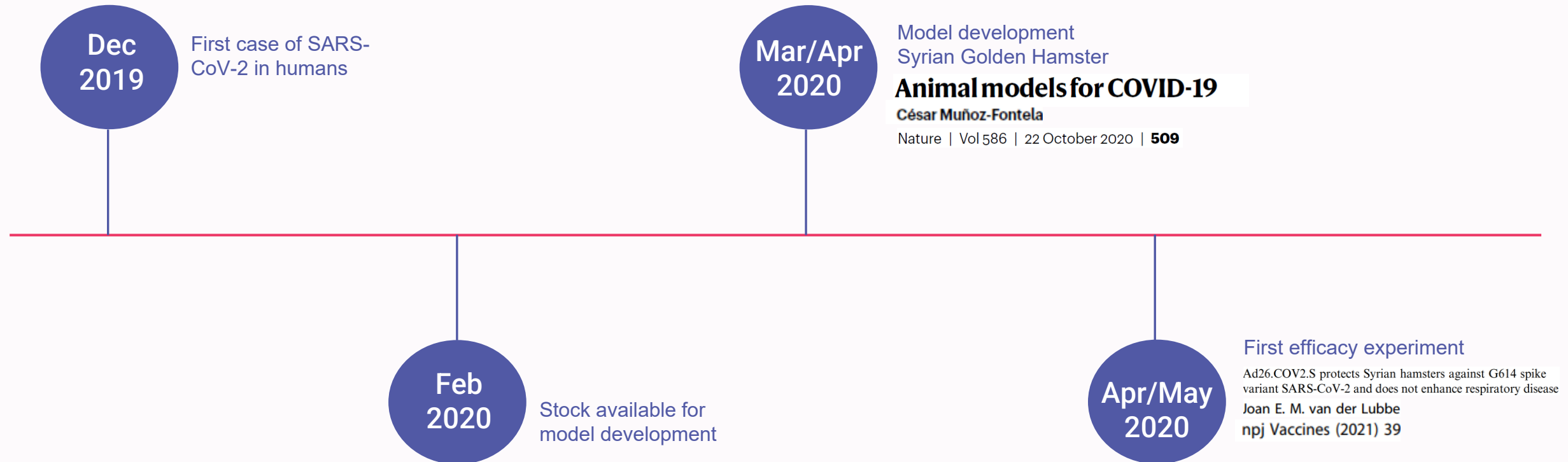
¹ CureVac AG

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 Huber¹, Aneesh Vijayan¹, Liesbeth Dekking¹, Ella van Huizen¹, Jessica Vreugdenhil¹, Ying Choi¹, Miranda R.M. Baert¹, Karin Feddes-de Boer¹, Ana Izquierdo Gil¹, Marjolein van Heerden², Tim J. Dalebout³, Sebenzile K. Myeni³, Marjolein Kikkert³, Eric J. Snijder³, Leon de Waal⁴, Koert J. Stittelaar⁵, Jeroen T.B.M. Tolboom¹, Jan Serroyen¹, Leacky Muchene¹, Leslie van der Fits¹, Lucy Rutten¹, Johannes P.M. Langedijk¹, Dan H. Barouch⁶, Hanneke Schuitemaker¹, Roland C. Zahn¹, Frank Wegmann¹

SARS-CoV-2 Model Development Timelines





Three R's

Reduction

Reduce animal testing and the number of animals used in experiments to a necessary minimum.

Replacement

As far as possible, animal experiments should be replaced by alternative methods.

Refinement

The test methods are used in such a way that the burden/discomfort on the animals is reduced to a minimum.



Three R's

Reduction

Reduce animal testing and the number of animals used in experiments to a necessary minimum.

Replacement

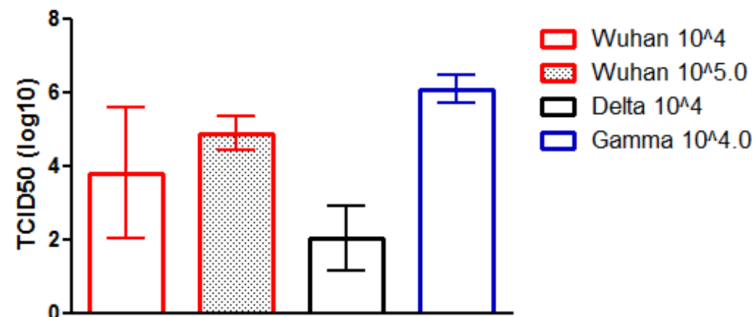
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Refinement

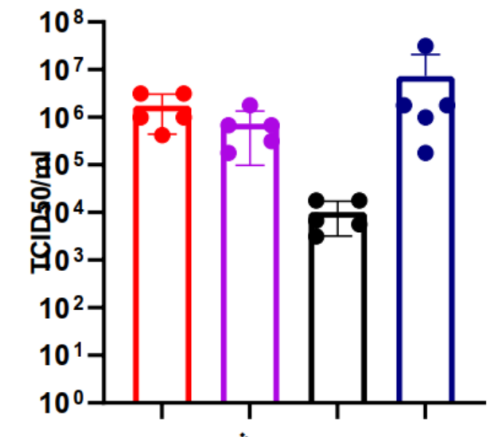
The test methods are used in such a way that the burden/discomfort on the animals is reduced to a minimum.

Ex vivo lung slices

Lung Slices day 4 post infection



Lung Tissue day 4 post infection





Three R's

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Replacement

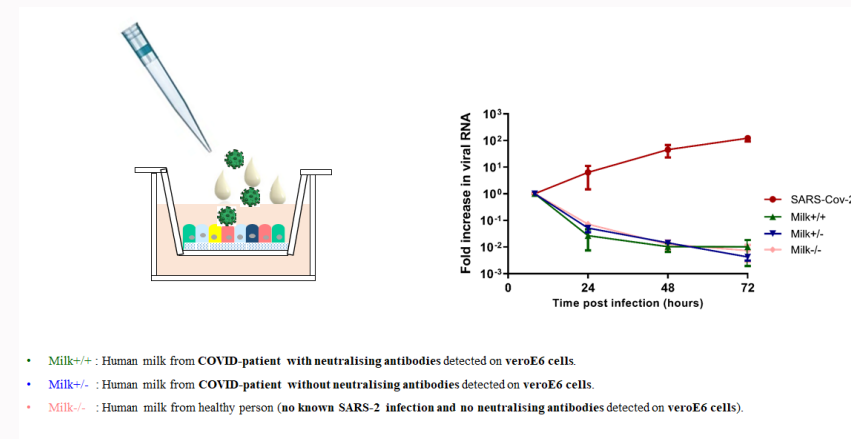
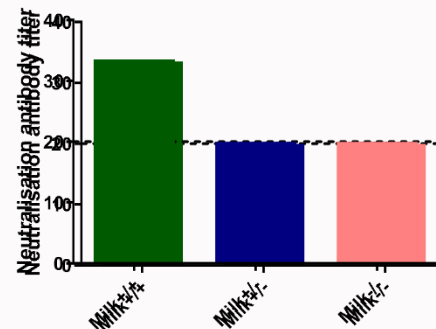
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Refinement

The test methods are used in such a way that the burden/discomfort on the animals is reduced to a minimum.

Organoids

Can human milk neutralize SARS-CoV-2





Three R's

Reduction

Reduce animal testing and the number of animals used in experiments to a necessary minimum.

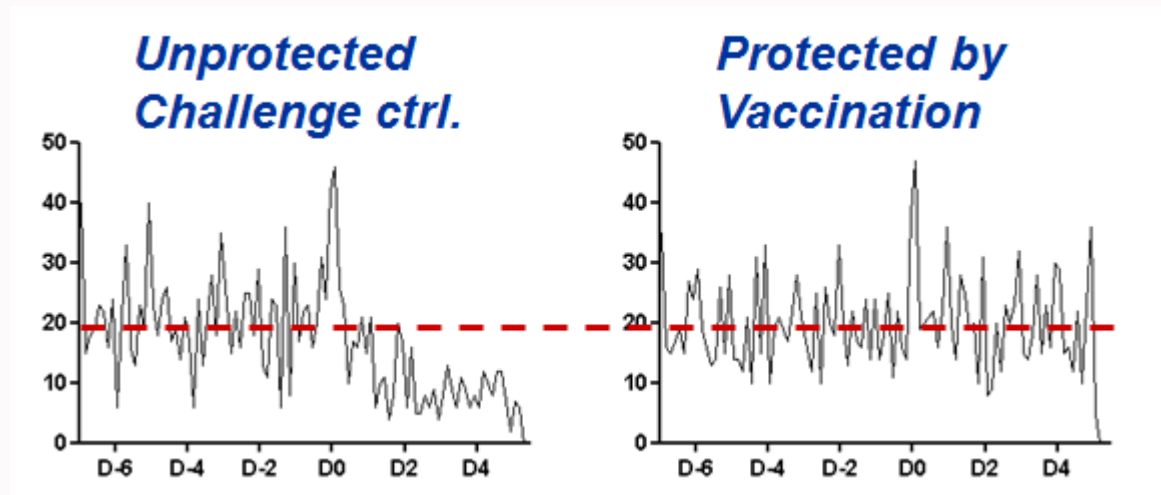
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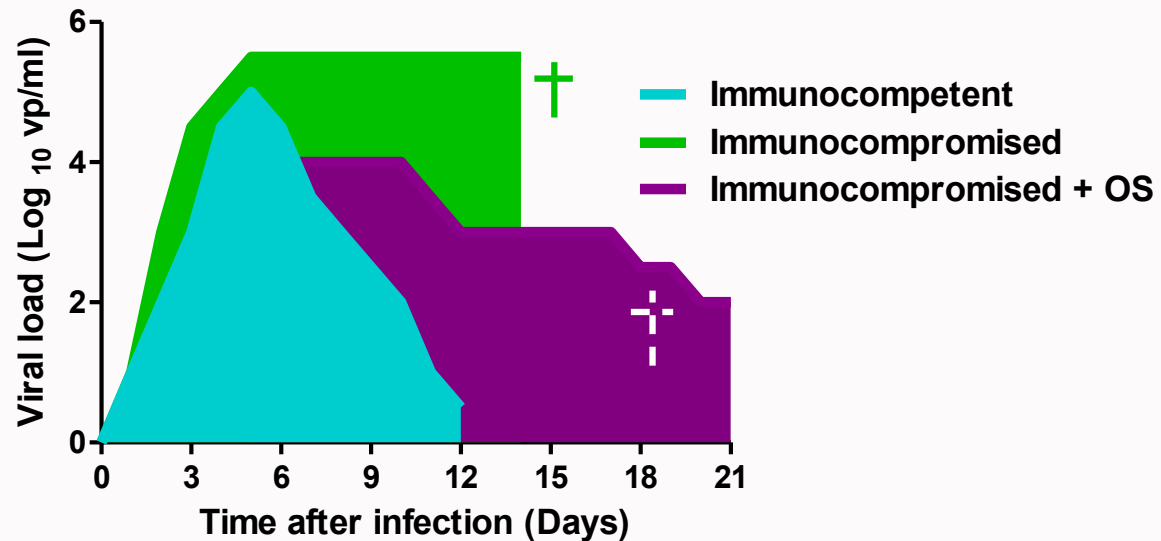
Advanced read-out techniques





Specialized preclinical models

Immunesuppression ferret models



Prolonged Influenza Virus Shedding and Emergence of Antiviral Resistance in Immunocompromised Patients and Ferrets

Erhard van der Vries¹, Koert J. Stittelaar², Geert van Amerongen², Edwin J. B. Veldhuis Kroeze², Leon de Waal², Pieter L. A. Fraaij^{1,3}, Roland J. Meesters^{4*}, Theo M. Luijckx⁴, Bart van der Nagel⁵, Birgit Koch⁵, Arnold G. Vulto⁵, Martin Schutten¹, Albert D. M. E. Osterhaus^{1,2*}

Ferrets as a Novel Animal Model for Studying Human Respiratory Syncytial Virus Infections in Immunocompetent and Immunocompromised Hosts

Koert J. Stittelaar^{1,*}, Leon de Waal¹, Geert van Amerongen¹, Edwin J.B. Veldhuis Kroeze^{1,2}, Pieter L.A. Fraaij², Carel A. van Baalen¹, Jeroen J.A. van Kampen², Erhard van der Vries^{2,3}, Albert D.M.E. Osterhaus^{1,2,3} and Rik L. de Swart²



Specialized preclinical models

HRSV ferret transmission model

Transmission of Human Respiratory Syncytial Virus in the Immunocompromised Ferret Model

Leon de Waal¹, Saskia L. Smits¹, Edwin J. B. Veldhuis Kroeze^{1,2}, Geert van Amerongen¹, Marie O. Pohl¹, Albert D. M. E. Osterhaus^{1,3} and Koert J. Stittelaar^{1,*}

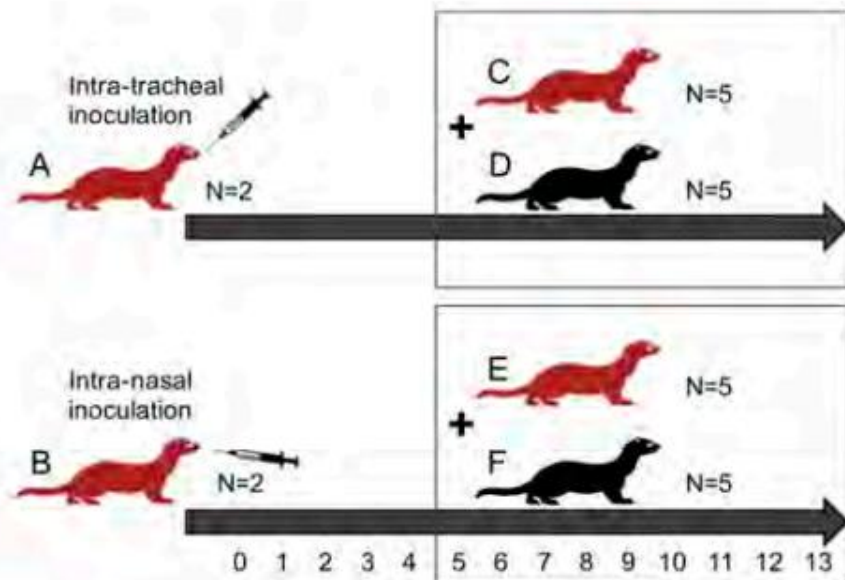


Table 2. Number of ferrets with throat and nose swabs positive for HRSV RNA and for replication-competent HRSV.

Ferret Group	Throat Swab RT-PCR	Nose Swab RT-PCR	Throat Swab VI	Nose Swab VI
A (N = 2)	2	2	2	2
B (N = 2)	2	2	2	2
C (N = 5)	4	3	4	2
D (N = 5)	4	4	4	3
E (N = 5)	4	2	4	2
F (N = 5)	5	4	3	1

Supporting Clinical Trials in Infectious Diseases with a One Stop Shop Solution.

Combining routine and specialty
testing in support of clinical trials

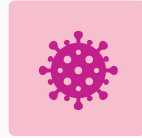


Infectious Diseases Highlights Harmonized With Our Virology Center Of Excellence



~600

ID trials since 2018



~250

COVID-19 trials



90%+

Trials include
Specialty Testing



CR (NL)

Virology center of
excellence

ID Indications Overview

Influenza (seasonal and pandemic
potential/zoonotic)

SARS1, MERS-CoV, SARS-CoV-2

HCV, HBV, HGV, HIV

RSV, hMPV, RV, PIV

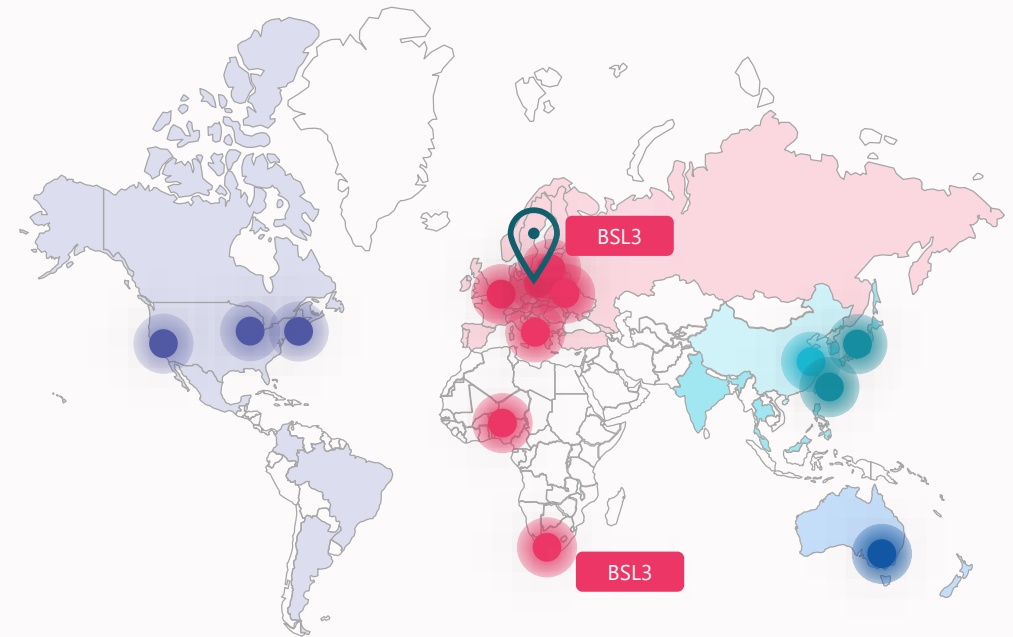
Dengue virus, Chikungunya Virus

Rabies, Polio virus

(Pulmonary aspergillosis,
C difficile, Tuberculosis)

And more...

Laboratories Overview



Main ID/virology laboratories globally with Cerba Research,
our virology center of excellence



A One-Stop Shop for ID Trial - Overall capabilities



Genomics

Microbiome
Metagenome
TCR / BCR Seq
qPCR/ddPCR
NGS/WGS/WES
RNA-Seq
Single-cell Seq
HLA genotyping

Routine/Safety

Serology testing
Virology testing
Sr calprotectin
Anti-IFN alpha-2
Hematology
Biochemistry
Coagulation
Urinalysis
Lipase
Amylase
Thyroid function

LBAs /CBAs/ VNAs

ELISA
HAI/ELLA
Virus neutralization
assays
MSD
Multiplex cytokine
profiling (37-plex)
50+ ligand binding
assays

Cell

Flowcytometry
Intracellular cytokine
detection by FCM
Immunophenotyping
Collection & processing of
PBMCs
ELISpot assays

Tissue

Biomarker analysis &
immune profiling
Multiplex/Simplex IHC
Full histopath service
(clinical & preclinical)
NanoString®
FISH
ISH



A One-Stop Shop for ID Trial - Overall capabilities => **ID focus**



Genomics

Microbiome
Metagenome
TCR / BCR Seq
qPCR/ddPCR
NGS/WGS/WES
RNA-Seq
Single-cell Seq
HLA genotyping

Routine/Safety

Serology testing
Virology testing
Sr calprotectin
Anti-IFN alpha-2
Hematology
Biochemistry
Coagulation
Urinalysis
Lipase
Amylase
Thyroid function

LBAs /CBAs/ VNAs

ELISA
HAI/ELLA
Virus neutralization assays
MSD
Multiplex cytokine profiling (37-plex)
50+ ligand binding assays

Cell

Flowcytometry
Intracellular cytokine detection by FCM
Immunophenotyping
Collection & processing of PBMCs
ELISpot assays

Tissue

Biomarker analysis & immune profiling
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NanoString®
FISH
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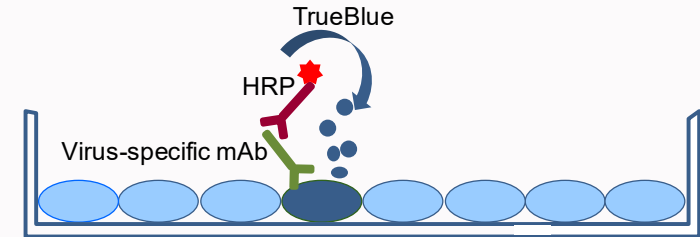
Specialty testing (SARS-CoV-2 as an example)

SARS-CoV-2 Serology and Virology



ViroSpot-based assays for measuring virus titer, virus neutralization, and inhibition of virus replication

- Cell-based assays for enumerating virus-infected cells:
Infected cells are detected via a virus specific mAb



- Automated detection and counting of plaques or virus-infected cells using CTL ELISpot reader (GLP and 21CFR part 11 compliant software)

Major advantage:

This type of assays use the wild type viruses → golden standard; no pseudo viruses or other surrogate systems needed



ViroSpot-based assays for SARS-CoV-2

- **Virus titration** (titer: TCID₅₀/mL)
 - Validated for nasopharyngeal (NP) swabs
 - Validated range: Virus titer 1.5 – 5.9 Log₁₀ TCID₅₀/mL
- **Neutralization assay** (titer: MNA₅₀/80) for testing serum and mAbs
 - Validated for SARS-CoV-2 Wuhan and all VoC
 - Validated range: MNA₈₀ titer 21 – 3,972
- **Antiviral assays** (IC₅₀)
 - SARS-CoV-2 Wuhan, VoC

Other assays for SARS-CoV-2

- **ELISA** (titer or Elisa Unit (EU)/mL)
- **MesoScaleDiagnostics** (MSD) platform
 - several V-plex and S-plex SARS-CoV-2 Panels and V-plex Respiratory Panel 1 (for the detection of 6 respiratory disease antigens)
- **Cytotoxicity assays** (LDH release, WST-8, Cell Titer-Glow)

Specialty testing (SARS-CoV-2 as an example)

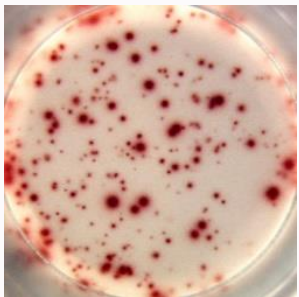
SARS-CoV-2 CMI (ELISPOT and Flowcytometry)



ELISpot On Demand

- Quantification of antigen-specific B and T cells at single cell level.
- Define the quality of the immune responses (e.g., Th1/Th2/Th17; Ig isotypes)
- To measure circulating ASCs and antigen-specific B cell memory
- Enzymatic- or fluorescence-based ELISpot
- Automated High-throughput analysis
- Assays are qualified for phase 1 and 2 clinical studies

Spots represent the ‘footprints’ of secreting cells



Cytokine secreting cells
(T cells)



Antibody secreting cells
(B cells)

ELISPOT / FLUOROSPOT Assay

Based on the commercial kits (e.g. Cellular Technology Limited).

Read-out by CTL UV X Analyzer and ImmunoSpot® software.

Assays have been qualified for phase 1 and phase 2 studies.

Multicolor flow cytometric analyses of SARS-CoV-2 specific T lymphocytes

Phenotype

- T cells subsets (e.g., CD4+, CD8+ T cells, Tregs, T follicular helper cells)
- Differentiation subsets (e.g., Naïve vs memory subsets)
- Activation markers (to identify antigen-specific responding cells)

Effector functions (Intracellular cytokine staining)

- Cytokines (IL-4/IL-6/IL-2/TNF- α /IFN- γ)
- Chemokines
- Cytotoxicity (e.g., Granzyme B)

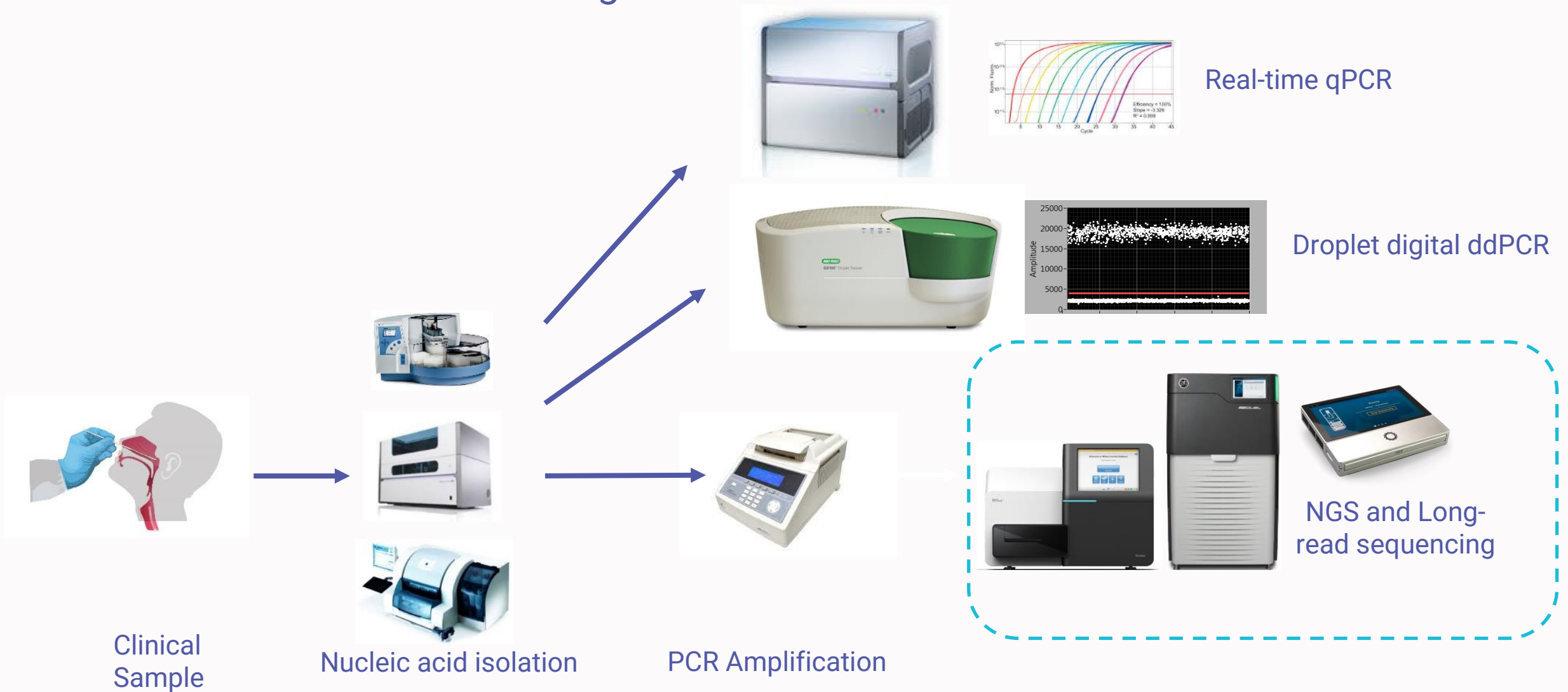
On demand panel design

Specialty testing (SARS-CoV-2 as an example)

SARS-CoV-2 Genomics



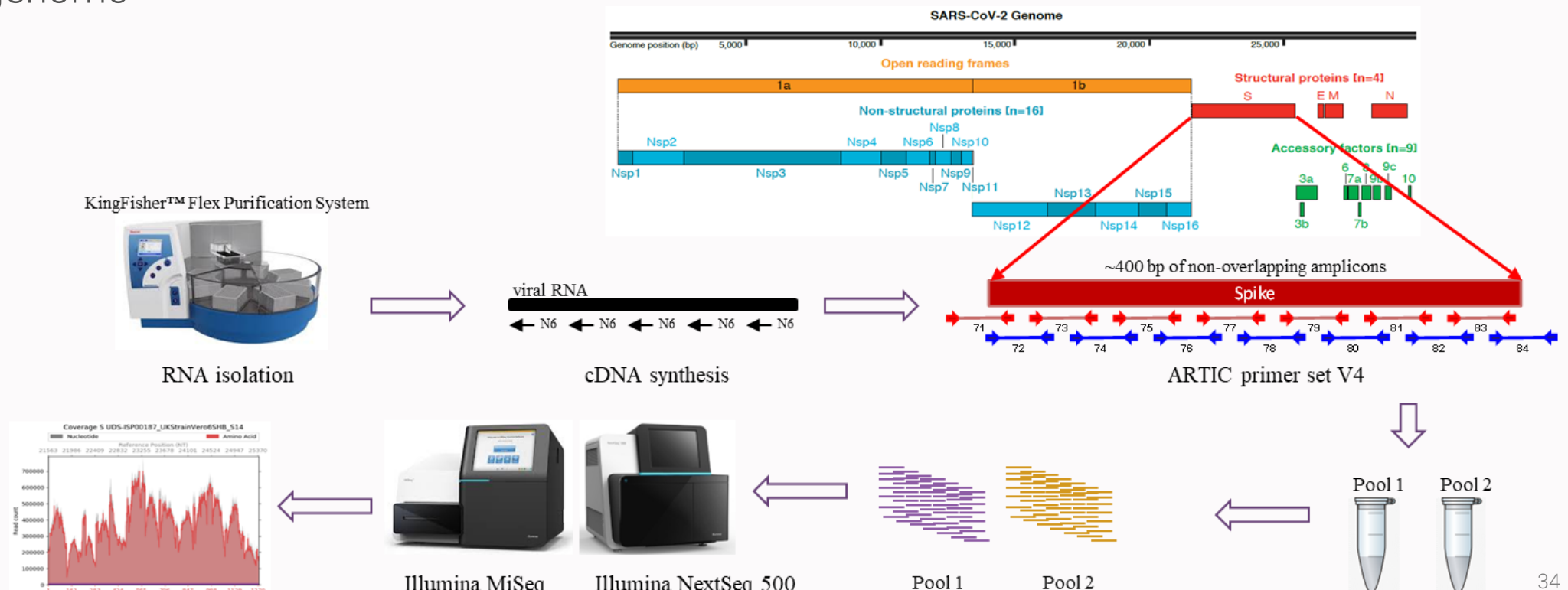
SARS-CoV-2 Molecular Testing





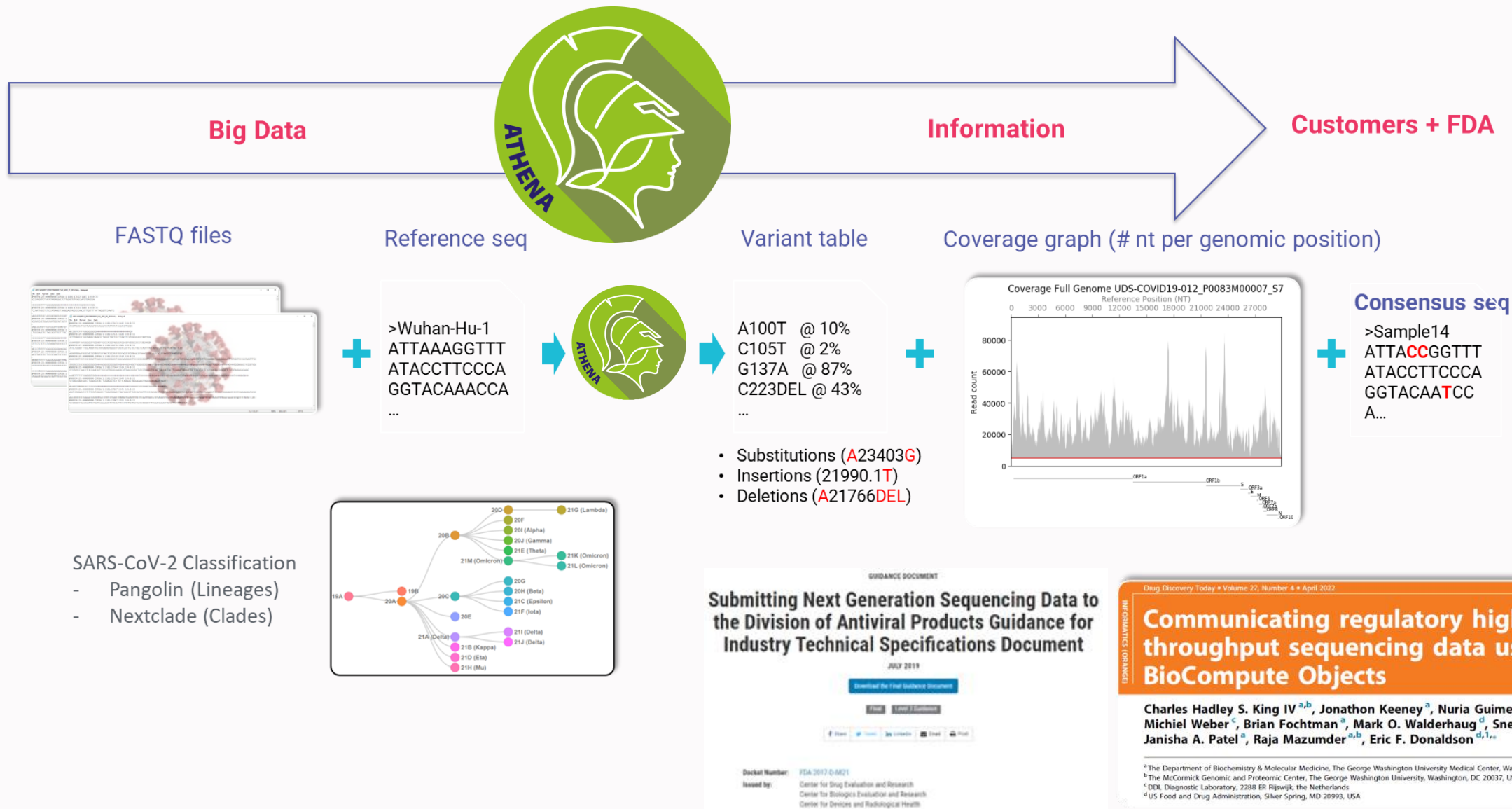
SARS-CoV-2 sequencing

- Sequencing of SARS-CoV-2 has been used to monitor circulating strains (lineage analysis), detect resistant variants for antiviral therapy and characterize breakthrough infections in vaccine studies
- Targets developed at the beginning of the pandemic:
 - Whole genome
 - Spike
 - RdRP
 - Nsp12





NGS Sequencing Data Flow: From Big Data To FDA Submission



Global Capabilities in CTO PBMC Isolation & Processing



Global and expertised Capabilities In Clinical Trial Operations

Local, Regional,
National, International



Global / International
Shipment



Spacial Conditions,
Incl. Dry Ice



Multiple Couriers
Worldwide



IATA / ADR



Support of global studies

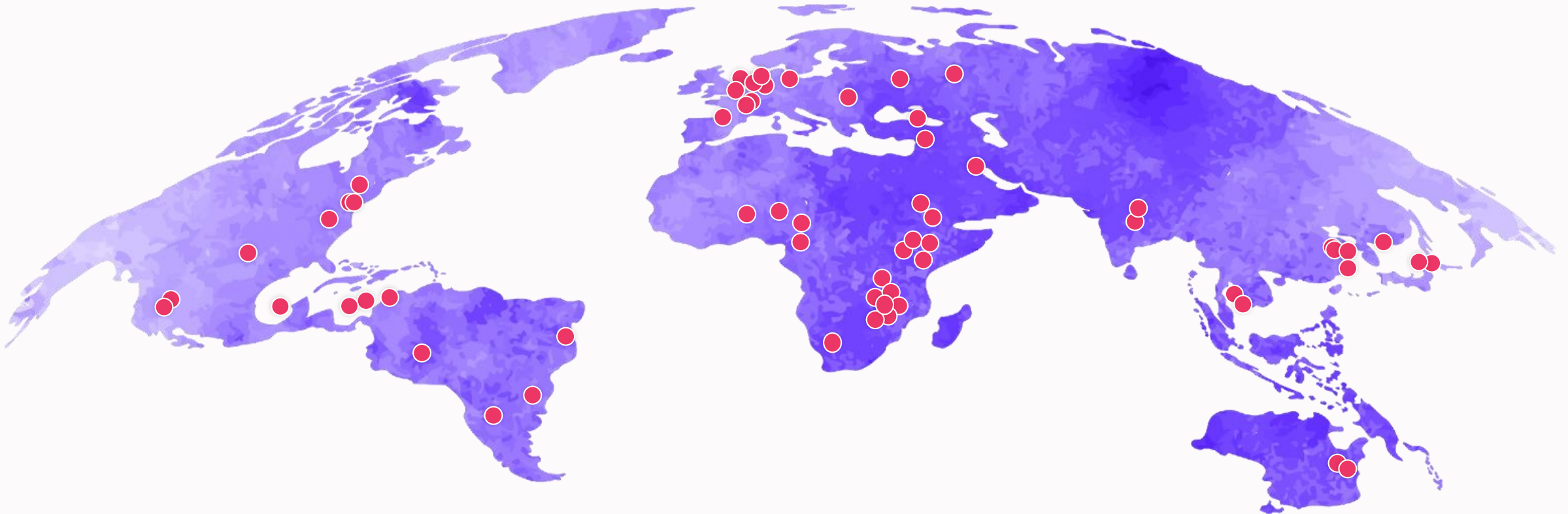
- Global logistic planning & support
- Customized design of sample kits per study and visits
- Building sample kit and (translating) lab manuals
- Pro-active and prospective reconciliation
- Contingency planning
- In-house biomedical products department
- Sample storage / Biobank
- [PBMC isolation & processing](#)
- Database management with QA and QC
- 24/7 helpdesk



PBMC Processing Capability & Expertise

45 processing labs in America,
Europe, Africa, Asia, and Australia

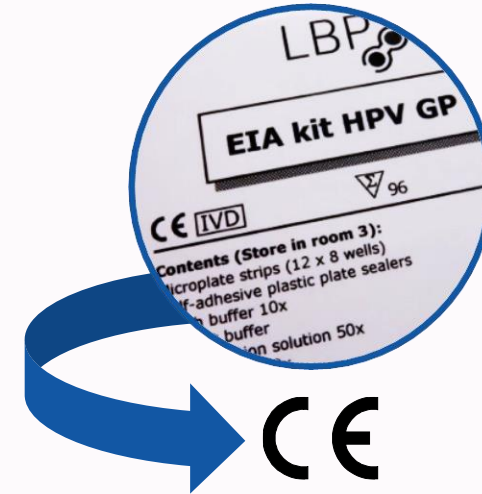
- > Premium Logistics
- > High Quality Standards
- > Extensive Network
- > Standardized SOP



- Diagnostic kits
- Clinical trial kits
- IVD directive 97/78/CE
- All classes
- Annex IIa and IIb (high risk, Notified Body-involvement)
- Annex III (self-certifying)
- Prepared for the new IVD directive
- Customized kits
- Prelabeled kits & Forms

Our Services

Production of **135.000** Kits Annually



CE



FDA
compliant



Cerba Research

A Global One stop Shop Solution Combining
Routine and Specialty testing in ID





Lessons learned and preparation for the next Pandemic

We deploy an expert global team who can utilise their experience, their knowledge and the best technologies available to accelerate clinical trials to ultimately reduce the burden of infectious disease

Technology & Infrastructure

BSL3 & BSL2 facilities

Largest global PBMC & processing lab network across 5 continents

Expertise in building bespoke virology test kits

Certified and qualified labs for animal studies and sample analyses

Science and knowhow

Certifications and permits in place – Coronavirus Animal models permits (SARS-1, MERS).

Readily available platforms and (animal) models -HPAI

Virospot assay broadly applicable, same primary Ab for CovS. Suited for (HP)AI

One stop shop: Same assay platforms preclinical and clinical studies if opportune

People & network

Scientist to scientist communication

Trained and certified team

Access to newest clinical isolates

Access to university insights (susceptibility SARS-2 VoC in animal models)

Access to serum (low-mid-high responders)

Pandemic Preparedness: From Proof of Concept to Clinic



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