Through a CRO’s Perspective:

Innovative, agile solutions in vaccine efficacy testing

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Necessity breeds innovation

The first step in developing a PNA is to produce the pseudotyped virus

Once the genetic sequence of the SARS-CoV-2 virus was released in early 2020, the race began to develop therapeutics and prophylactic vaccines against the virus. Vaccine manufacturers worldwide shifted their R&D focus concurrently. SARS-CoV-2 quickly proved to be both highly transmissible and virulent, deeming a BSL-3 facility necessary to handle the live virus, whether to produce a vaccine or construct assays. For the first time in vaccine history, more than 100 candidates were in development against the same pathogen simultaneously. The demand for efficacy testing created by the SARS-CoV-2 vaccine development projects outpaced what the limited BSL-3 facilities could provide.

While wild-type virus neutralization assays are considered the gold standard for measuring levels of neutralizing antibodies, they are low throughput and require a high level of containment for highly pathogenic viruses. IQVIA Laboratories responded to the industry demand by developing a pseudotyped neutralization assay for SARS-CoV-2, and other assays necessary to measure humoral and cellular responses to the virus and its variants. The molecules used in IQVIA Laboratories' qualified and validated assays closely resemble those of the SARS-CoV-2 virus, but lack the viral replication gene necessary to be infectious. This allows the assays to be performed in BSL-2 laboratories at high throughput.

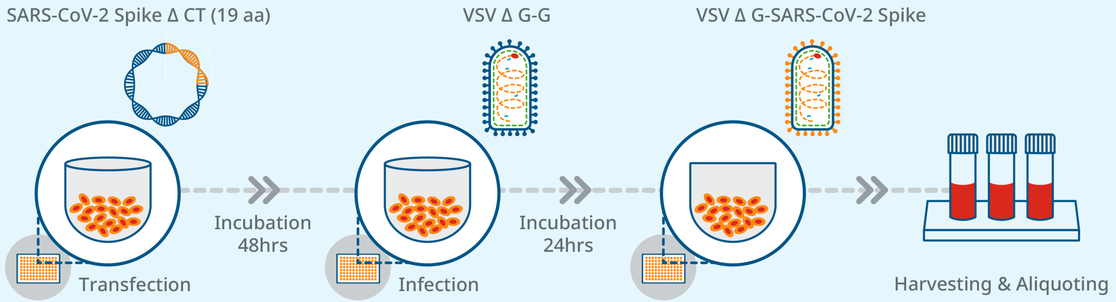
Constructing a robust assay

Pseudotyped virus production

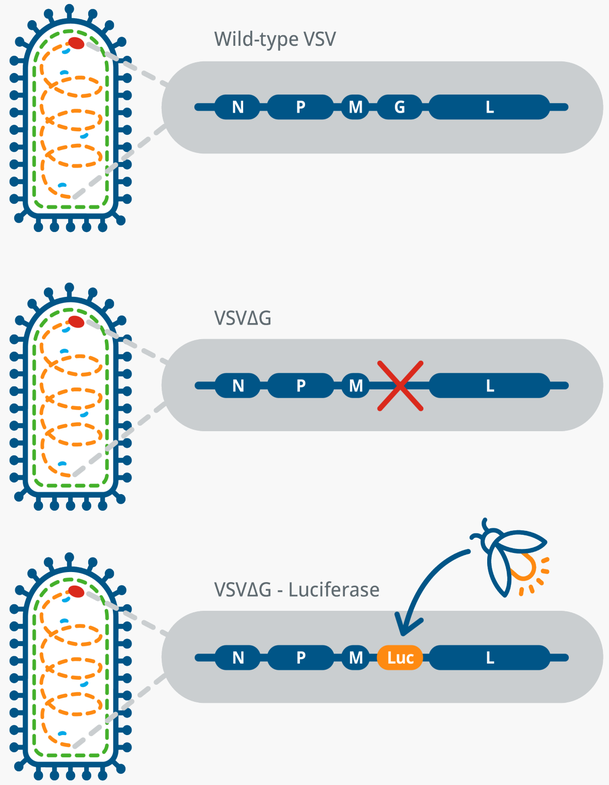
IQVIA Laboratories' Protein Science team used modified VSV virus, where its glycoprotein (G) was replaced by firefly luciferase reporter gene (VSVΔG), to generate the pseudotyped virus. ES293 cells were transfected with a plasmid containing the SARS-CoV-2 spike glycoprotein gene. The transfected cells, expressing the SARS-CoV-2 spike on their surface, were then infected by the recombinant VSVΔG. The pseudotyped viruses (VSVΔG-SARS-CoV-2 Spike) were released from the infected cells harboring the SARS-CoV-2 spike.

These pseudotyped viruses are not able to produce infectious progeny viruses. They are neutralized by antibodies just like the wild-type virus, but lack the gene for viral replication. This genetic alteration made the pseudotyped virus viable for use in BSL-2 facilities. Using the luciferase reporter allows luminescence to be measured by a luminometer, significantly increasing the speed and accuracy of data analysis.

MODIFICATION OF THE VSV VIRUS



MODIFICATION OF THE VSV VIRUS



Constructing a robust assay

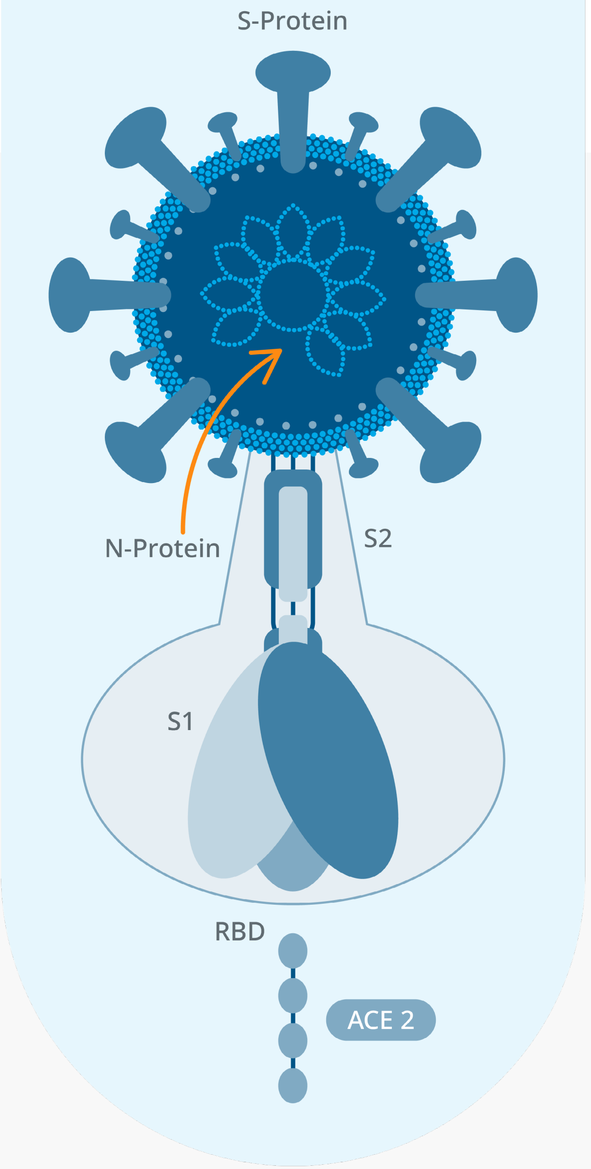
Development of critical reagents

Unique for a contract research organization (CRO), IQVIA Laboratories boasts an in-house Protein Sciences team that works in close collaboration with their Assay Development and Clinical Operations team. The collaboration between the Clinical team and Protein Sciences team enables IQVIA Laboratories to produce customized and fully characterized reagents without reliance on a third-party vendor or commercial availability.

In support of the studies associated with SARS-CoV-2, the Protein Sciences team optimized the key antigen productions (Spike antigen (S), Receptor Binding Domain (RBD), and the Neucleocapsid (N)). They also optimized the pseudotyped virus production strategy to enable large-scale production of robust reagents required to support the ever-growing demand for efficacy testing against SARS-CoV-2 variants. This rigorous protein expression process is monitored according to strict acceptance criteria and ensures reproducible and stable pseudotyped virus.

Once all initial criteria is met for a lot of pseudotyped virus, it qualifies for head-to-head technical bridging in PNA tests. Technical bridging occurs when the new lot is tested and analyzed against a reference batch. If the bridging is deemed statistically acceptable, the new lot is approved for use in clinical trials.

In addition, the pseudotyped virus and other particles are stress tested, and their long-term stability is assessed periodically to ensure reliability in the PNA results.



Constructing a robust assay

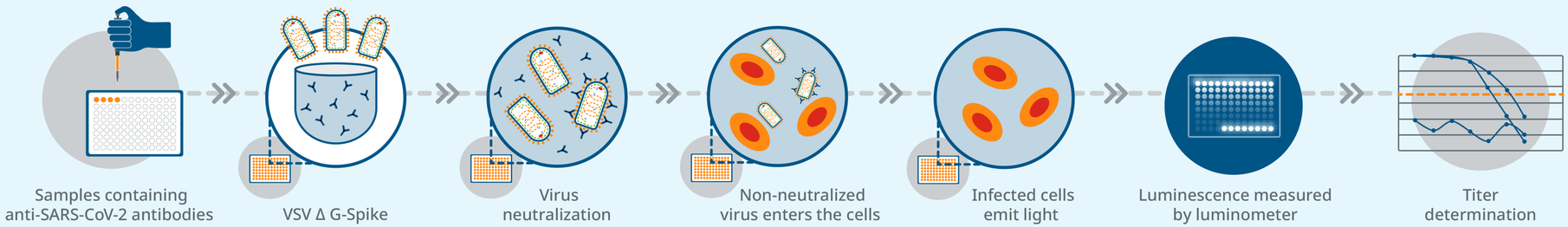
Pseudotyped neutralization assay

Once the pseudotyped virus was successfully produced, the next task was to develop a robust assay to assess immunogenicity. IQVIA Laboratories' pseudotyped neutralization assay (PNA) is similar to the microneutralization assays (MNA) being performed at BSL-3 and -4 facilities with live virus. Pre-clinical or clinical sera samples containing anti-SARS-CoV-2 antibodies are diluted. The VSVΔG-SARS-CoV-2 spike is added, and the spike is neutralized by the antibodies. This complex is incubated with Vero E6 cells, non-neutralized virus enters the cells via the ACE2 receptor, and cells start expressing the luciferase. The luciferase substrate is added and cell lysis occurs. The infected cells emit light, and a luminometer is used to quantify the level of light per well. The level of light emitted is inversely proportional to the level of neutralization by the antibodies. A regression is used to determine titer.

Accuracy and precision

To demonstrate the robustness of the PNA, IQVIA Laboratories participated in Duke University's initiative, SARS-CoV-2 Neutralization Assay Concordance Survey (SNACS). COVID-19 convalescent serum samples with high, medium, and low titers were tested, where samples were tested in triplicate to assess precision and accuracy. The results clearly demonstrated that IQVIA Laboratories' assay is best in class, and confirmed its high concordance with live-virus assays.

PSEUDOTYPED NEUTRALIZATION ASSAY



Constructing a robust assay

Assays for humoral and cellular response

In addition to the PNA, IQVIA Laboratories' SARS-CoV-2 portfolio includes assays to address primary and secondary clinical endpoints. Three ELISA assays were developed to assess the humoral response in its entirety. The ELISA-S and ELISA-RBD assess antibodies that target the entire Spike antigen and the Receptor Binding Domain respectively, which have been the most popular targets of vaccines. The ELISA-N assesses antibodies targeting the Nucleocapsid, and was developed to assess pre-exposure to SARS-CoV-2, an important differentiator in determining vaccine immunogenicity.

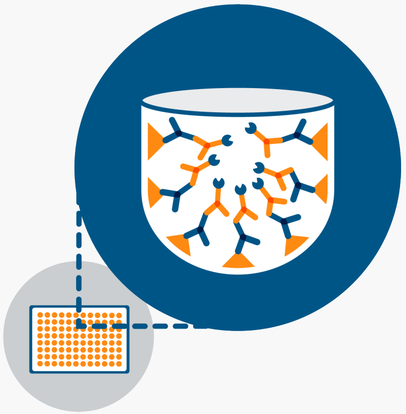
A quantitative reverse transcription PCR (RT-qPCR) assay measures viral shedding and ensures safety in vaccines using a hybrid or live attenuated virus.

ELISpot and flow cytometry assays were developed to assess the cellular response induced following immunization with SARS-CoV-2 Spike vaccines. Intracellular cytokine staining (ICS) utilizes flow cytometry, scanning for chemical messengers released by vaccine-induced T-cells.

The ELISpot specifically measures IFN-γ (Th1) and IL-5 (Th2) responses. Protective Th1-related cytokines are involved in cellular immune responses, whereas Th2-related cytokines are associated with humoral immunity and anti-inflammatory properties. The Th1/Th2 responses have been extensively assessed for the development of vaccines against viral pathogens to ensure that a vaccine does not contribute to a cytokine storm. As such, the ELISpot assay is required to assess safety of new vaccine candidates.

While stimulating both a humoral and cellular response is desirable for optimum protection against SARS-CoV-2, an overproduction of antibodies and/or cytokines can be detrimental, if not lethal, to an individual. IQVIA Laboratories' validated assays are used to ensure appropriate immune responses are triggered by the vaccines, while providing the necessary results to ensure its safety.

CELLULAR ASSAYS USED TO EVALUATE THE LEVEL OF FUNCTIONALITY		PHENOTYPING MARKERS	POLARIZATION OF THE RESPONSE(S)
CD154	IL-4	CD3	Th1
CD137	IL-5	CD4	Th2
Granzyme B	IL-13	CD8	Th17
IFN-γ	IL-17		
IL-2	TNF-α		



Foundational strategic partnerships

The assays IQVIA Laboratories and UKHSA developed have been published in nature protocols

United Kingdom Health Security Agency (UKHSA)

Prior to the pandemic, in 2019, IQVIA Laboratories and the UK Health Security Agency (UKHSA) recognized their complementary capabilities in development, qualification, and validation of biological assays, supporting deployment of vaccines. Together they benefited from deep scientific expertise, BSL-3/BSL-4 containment capabilities, and high throughput testing. When it came time to develop reagents and key assays to measure the humoral and cellular response to SARS-CoV-2 and vaccine candidates, IQVIA Laboratories' and UKHSA's established relationship became an undeniable asset. The immunological tools that IQVIA Laboratories and UKHSA developed have been used to support vaccine manufacturers through development and commercialization of prophylactic vaccines, ongoing evaluation of the efficacy of existing vaccines on the different SARS-CoV-2 variants, and policy decisions worldwide.

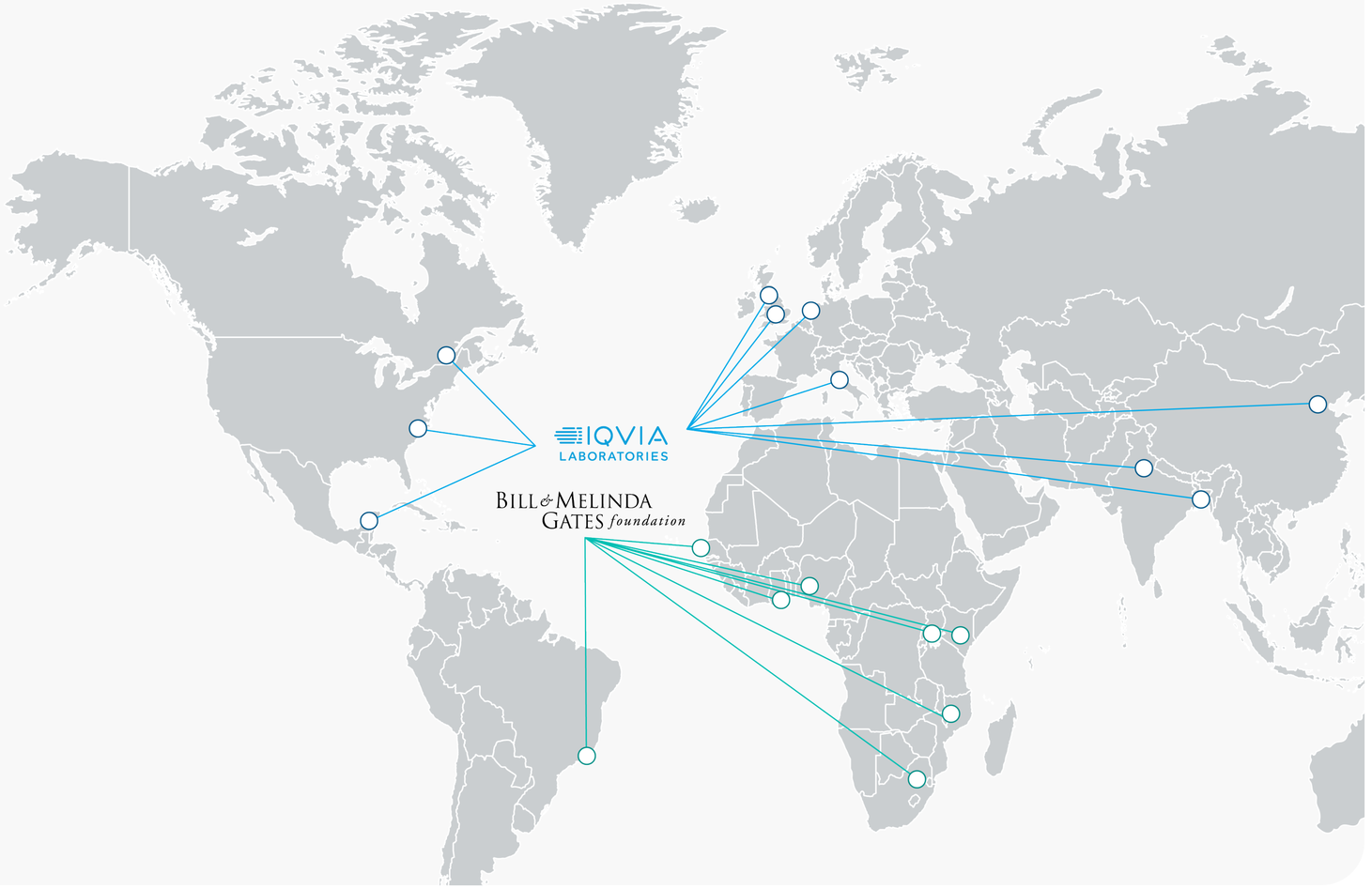
Bill & Melinda Gates Foundation (BMGF)

Early in the race to develop a prophylactic vaccine against SARS-CoV-2, the Bill & Melinda Gates Foundation (BMGF) had more than 30 grantees with vaccine candidates in development. Each grantee used their own assays, which posed a significant problem for assessing and comparing efficacy of these vaccines. Building on a previous successful collaboration, BMGF partnered with IQVIA Laboratories to standardize efficacy testing for the grantees. As the number of BMGF grantees developing SARS-CoV-2 vaccine candidates grew, IQVIA Laboratories and BMGF extended their partnership. IQVIA Laboratories now transfers their assays to various labs within the BMGF network to support preclinical and clinical studies.

Foundational strategic partnerships

Coalition for Epidemic Preparedness Innovation (CEPI)

As the pandemic spread, the volume of development projects using different methods, reagents, and data collection and analysis tools quickly made the prospect of comparing immune response and efficacy of therapeutics impossible. In an effort to standardize immunological assays and protocols, and facilitate rapid evaluation, approval, and dissemination of the most effective vaccine candidates, the Coalition for Epidemic Preparedness Innovation (CEPI) created a Centralized Laboratory Network. IQVIA Laboratories and UKHSA were identified as two reference laboratories for the network, and would be relied upon to develop and transfer qualified and validated assays and key reagents necessary for immunogenicity and efficacy evaluation to the network labs worldwide. This unprecedented collaboration and harmonization between historical competitors revolutionized the industry's ability to respond rapidly to the SARS-CoV-2 pandemic, as well as to new emergent diseases.



Scale to support late phase clinical testing

Addressing variants of concern

The high transmission rate and mutagenetic nature of SARS-CoV-2 has created a persistent problem for public health: Variants of Concern (VOCs) and Variants of Interest (VOIs). To study the efficacy of a vaccine against each of the variants requires adaptation of the testing process, and the production of a new pseudotyped virus based on the genetic make-up of each VOC. With each new variant that occurs, the convalescent sera used in the assays must be reassessed. IQVIA Laboratories and UKHSA assess them first in the wild-type, and then creates a new pseudotyped virus as needed for use in the PNA. Their results are shared back to vaccine manufacturers, who use efficacy data of their vaccine candidate against new VOCs/VOIs to inform decisions regarding the modification of formulation and/or dosing to ensure no immune escape.

STAGES OF CLINICAL TRIALS

As SARS-CoV-2 continues to mutate, and vaccine developers increase the number of participants in their trials as studies progress to late phase clinical, efficacy testing demand and complexity increases exponentially. IQVIA Laboratories has responded by identifying points of redundancy and automating steps to maximize efficiency, while maintaining the highest standards of clinical testing.

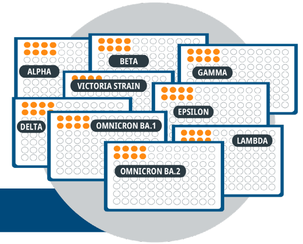
Automation

IQVIA Laboratories has purposefully selected steps of their assays to automate, which increases testing capacity to 10,000 tests per month to support clinical trials. To date, IQVIA Laboratories has generated more than 250,000 test results. Steps that have been fully automated include:

- **Dispense media in pre-dilution plates**
- **Sample serial dilution**
- **Addition of pseudotyped virus**

The effectiveness of this automation has been thoroughly assessed. Every automated step is evaluated to verify that the automation itself has not impacted the quality of the results being generated. By performing the assays as they were initially designed, as well as with the integration of robots, and using the same sera panels, IQVIA Laboratories has demonstrated high correlation of results between the manual and automated systems.

Variant list (VOC/VOI)



VARIANT	LINEAGE	ORIGIN	PSEUDOTYPED VIRUS	ANTIGEN
D614	NA	China	X	X
RBD	NA	China		X
Nucleocapsid	NA	China		X
D614G	B.1	Multiple Countries	X	X
Alpha	B.1.1.7	UK	X	X
Beta	B.1.351	South Africa	X	X
Delta	B.1.617.2	India	X	X
Delta+	B.1.617.2 + K714N	India	X	X
Epsilon	B.1.429	USA/California	X	X
Eta	B.1.525	Multiple Countries	Ongoing	
Gamma	P.1	Brazil	X	X
Iota-1 & 2	B.1.526	USA/NY (1 & 2)	X	X
Kappa	B.1.617.1	India	Ongoing	Ongoing
Not yet classified	B.617.3	NA	X	
Lambda	C.37	Peru	X	
Mu	B.1.621	Colombia	X	
Omicron	BA.1/B.1.1.529.1	South Africa	X	X
Omicron	BA.2/B.1.1.529.1	Multiple Countries	Ongoing	Ongoing
Theta	P.3	Philippines	X	
Zeta	P.2	Brazil	X	X
Not yet classified	A.VOI.A2	NA	X	
Not yet classified	AY.1	NA	X	
Not yet classified	C.1.2	NA	X	

Application of IQVIA Laboratories' SARS-CoV-2 assays

The global health crisis caused by the SARS-CoV-2 virus has raised questions only answerable with considerable data. IQVIA Laboratories' robust cellular and humoral assays have generated vast amounts of reliable data for a significant breadth and depth of studies. IQVIA Laboratories has generated data for studies determining reactogenicity and immunogenicity of booster vaccination,[1] as well as vaccine efficacy against ancestral and novel variants of SARS-CoV-2. Their assays have been used to evaluate safety and efficacy of SARS-CoV-2 vaccines in various populations such as children, adults, adults 65 years of age and older, and adults with comorbidities. And the evaluation of prophylactic and therapeutic effects of monoclonal antibodies was done with IQVIA Laboratories' technology. The data generated by the extensive use of IQVIA Laboratories' assays has contributed to bringing effective vaccines to market, as well as informed and supported a number of policy decisions worldwide.

Through the collaboration made possible by CEPI's Centralized Laboratory Network and the grantees of BMGF, the assays and pseudoparticles developed at IQVIA Laboratories have been used in more than 100 vaccine development projects worldwide. IQVIA Laboratories has demonstrated reliable efficacy testing agnostic of vaccine modality. Their assays have generated data to support preclinical and clinical studies using mRNA, viral-vector, protein-adjuvant, and plant-derived virus-like particle vaccines.[2-3]

The rapid transmission rate and high virulence of the SARS-CoV-2 virus demanded a change in the way the industry develops vaccines and anti-viral therapeutics. Previously, typical vaccine development timelines have averaged 10 years. Astonishingly, the time from the SARS-CoV-2 genetic sequence's release to the submission of the first vaccine clinical trial data for regulatory review was a mere 314 days. IQVIA Laboratories' foresight to develop and deploy assays prior to being approached by a vaccine manufacturer, and their willingness to collaborate with historical competitors, enabled speed through critical development phases and an unprecedented response to the SARS-CoV-2 pandemic.

1. Munro AP, Janani L, Cornelius V, Aley PK, Babbage G, Baxter D, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicenter, randomized, controlled, phase 2 trial. *Lancet.* 2021;398:2258–2276. https://doi.org/10.1016/S0140-6736(21)02717-3.

2. Ward BJ, Gobeil P, Séguin A, et al. Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. *Nat Med.* 2021;27:1071–1078. https://doi.org/10.1038/s41591-021-01370-1.

3. Gobeil P, Pillet S, Boulay I, Séguin A, Makarkov A, Heizer G, et al. Phase 2 Randomized Trial of an AS03 Adjuvanted Plant-Based Virus-Like Particle Vaccine for Covid-19 in Healthy Adults, Older Adults, and Adults with Comorbidities. *medRxiv.* 2021;05.14.21257248. https://doi.org/10.1101/2021.05.14.21257248.

About IQVIA Laboratories

IQVIA Laboratories, the laboratory business of IQVIA, is a global leader in drug discovery and development laboratory services, offering a comprehensive suite of central laboratory and specialty biomarker services. Committed to scientific rigor and operational excellence, we support every phase of the drug discovery and development process across diverse regions and regulatory frameworks, ensuring the highest standards of data integrity and accelerating the delivery of transformative therapies to patients.

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