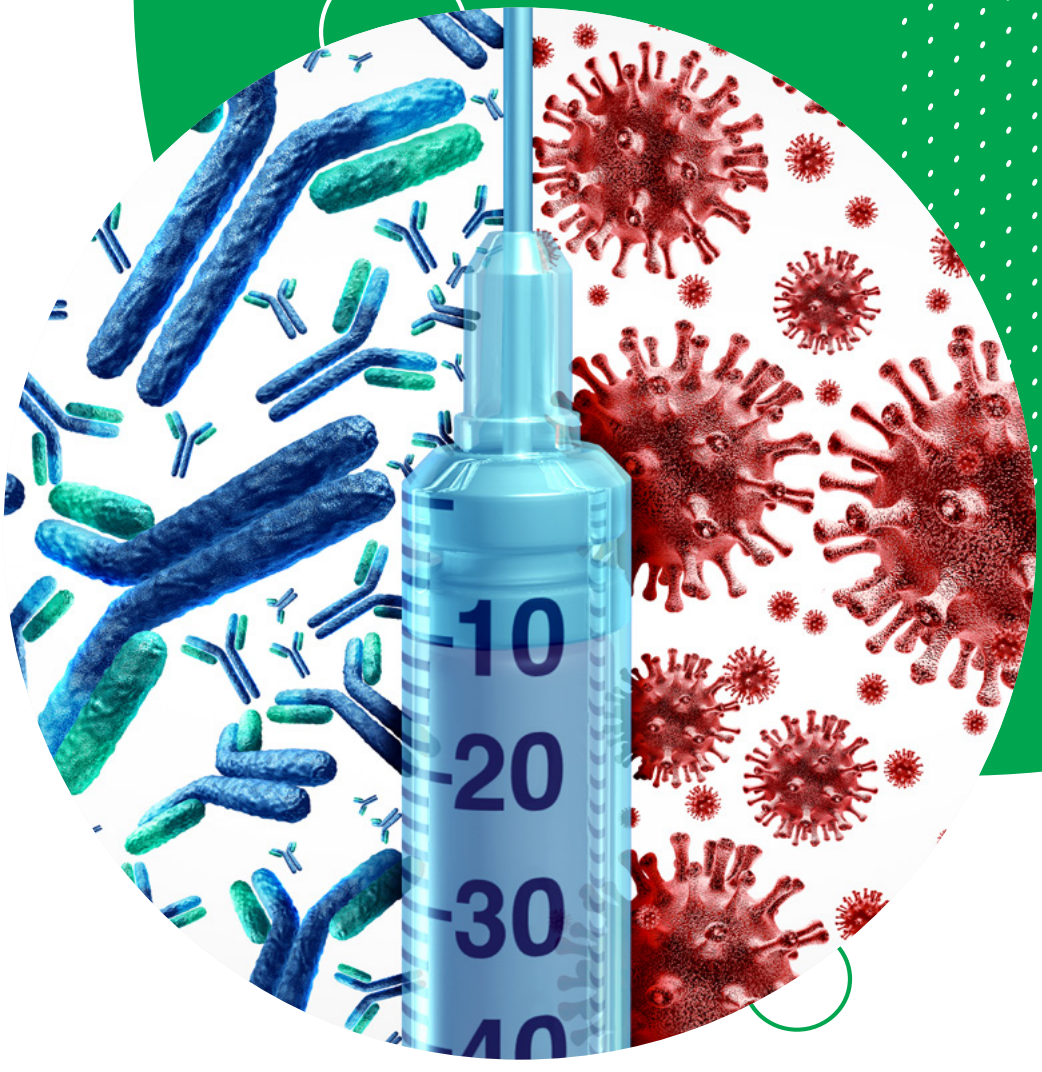
THE VACCINE JOURNEY: FROM BENCH TO NEEDLE



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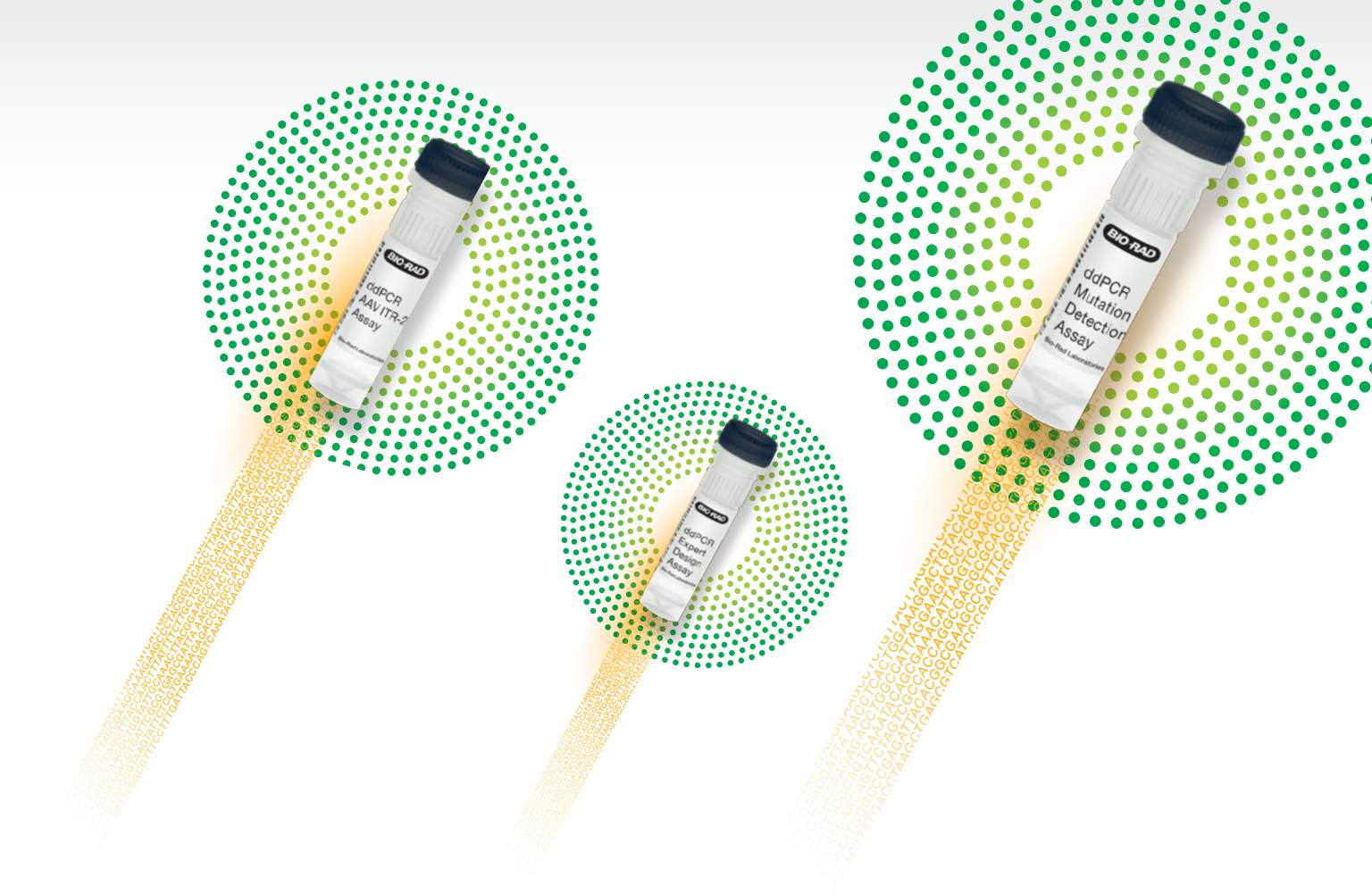

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THE WIDE WORLD OF VACCINES

In 1796, Edward Jenner performed the first vaccination against smallpox. After observing that English milkmaids who caught cowpox seemed immune to the related smallpox virus, Jenner inoculated an 8-year-old boy with pus from a cowpox lesion. With his immune system primed, the boy remained healthy when Jenner exposed him to infectious smallpox material several weeks later.[1]

Thankfully, vaccine technology has come a long way from its experimental beginnings. After Jenner's initial experiments, other scientists, including Louis Pasteur, soon developed various types of vaccines for different diseases.[1] Vaccine development flourished in the 20th century, and researchers continue to innovate today as novel infectious diseases present new challenges.

Whole Organism Vaccines

Many old and new vaccines consist of a weakened form of a disease-causing pathogen. Researchers develop these live attenuated vaccines (LAVs) by serially passaging the infectious microbe in cell culture, oftentimes on non-native host cells. During this process, mutations accumulate that render the microbe ineffective in its original host. Using a whole attenuated organism as vaccine material induces a robust response that mimics a real infection, as both the humoral and cellular immune systems respond to the foreign agent and develop memory B and T cells.[2] However, these vaccines may elicit an adverse reaction in immunocompromised individuals.

There have been rare instances of LAVs reverting back to pathogenic forms.[3] To avoid these events, scientists developed inactivated vaccines made from killed versions of disease-causing microbes. After growing infectious agents in cell culture, vaccine developers destroy them by chemical, heat, or radiation exposure. Because all parts of the virus or bacteria are present, inactivated vaccines induce robust antibody responses. However, most do not induce T cell responses because the killed pathogens do not divide within immunized individuals. Booster vaccine doses help strengthen the immune response, and scientists often formulate inactivated vaccines with adjuvants that activate the T cell response.[4]

Subunit Vaccines

Vaccines made from antigenic parts of pathogens, such as proteins or polysaccharides, are often more stable than whole pathogen vaccines. Subunit vaccines are suitable for immunocompromised and elderly people, although some of these vaccines require adjuvants and boosters as they may only induce antibody-mediated immunity.[3] Polysaccharide-based vaccines, such as the *Haemophilus influenzae* type B (Hib) vaccine, often require carrier molecules covalently linked to the pathogen's polysaccharides to help elicit a sufficient immune response.[3]

Developing subunit vaccines is more complex than developing those from whole pathogens. For example, protein-based vaccines require researchers to grow and purify protein from the pathogen itself or create a recombinant virus or bacteria that expresses the desired antigen. Some of these proteins, such as those found in HPV vaccines, must self assemble into virus-like particles (VLPs) that house the antigen on the surface.[5]

Viral Vector

Viral vector vaccines are made of a non-pathogenic virus that delivers genetic material coding for an immunogenic antigen to host cells. Using that genetic material, cells express the antigen, which mimics natural infection and elicits strong antibody and T cell responses. Scientists commonly use adenoviruses as delivery vehicles because of their high transduction efficiency, ability to grow to high titers in the laboratory, and ability to infect a variety of cell types.[6]

Nucleic Acid

Similar to viral vector vaccines, nucleic acid vaccines deliver an antigen's DNA or RNA code, which host cells use to build immunogenic proteins that stimulate both humoral and cellular immunity.[7] Nucleic acid vaccines are relatively simple and inexpensive to produce. Once an antigen's sequence is known, making a gene construct that codes for a desired antigen is more straightforward than purifying recombinant protein or inactivating or attenuating whole pathogens. mRNA vaccines, such as those produced to combat the COVID-19 pandemic, are typically encased in lipid nanoparticles, which facilitate absorption into host cells.[8] Recently, researchers explored delivering DNA vaccines consisting of high-expression plasmids via lipid nanoparticles like those used for mRNA vaccines and found increased efficacy.[9] Both DNA and RNA are somewhat immunogenic in nature, making them attractive materials for future vaccine efforts.

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OPTIMIZED R&D

From the lab bench through clinical trials, new vaccine development can take five to ten years, although the COVID-19 pandemic has shown what is possible on an accelerated timeline. Basic discovery research, preclinical animal studies, and human clinical trials all require researchers to carefully analyze their chosen vaccine material and the immune response of living subjects.

Identify an Antigen

Many compounds that make up a pathogen can trigger a host's immune system, but the ideal vaccine candidate is an antigen that induces robust, protective humoral and cellular responses. To identify promising vaccine candidates, researchers must understand an antigen's structure and its interaction with the host.

Researchers developing vaccines identify antigens that induce protective immunity by assessing the immune response of patients who have been infected by the pathogen of interest. For example, scientists analyze antigen interactions with neutralizing antibodies from patient serum samples using flow cytometers such as the Bio-Rad ZE5 Cell Analyzer. At the start of the COVID-19 pandemic, researchers used the ZE5 Cell Analyzer to confirm binding of the SARS-CoV-2 spike protein—the antigen central to COVID-19 vaccine development—to patients' neutralizing antibodies.[1]

Antigens are often under selective pressure, which generates variants that affect the immune response differently. To detect known single nucleotide variants and discriminate between closely-related viral or bacterial strains, researchers can employ Droplet Digital PCR (ddPCR). The Bio-Rad QX200 and QX ONE ddPCR systems do this by making thousands of measurements within a partitioned sample with high precision and low bias. This technique has proven essential during the COVID-19 pandemic for tracking variants within populations.[2]

Verify Expression

Once researchers identify a candidate antigen, vaccine development can begin. Assays such as SDS-PAGE and western blot help researchers assess proper antigen expression, whether from a viral vector vaccine that expresses the antigen within the host or for a protein-based vaccine that requires expression in cell culture. Bio-Rad's Stain-Free western blotting assists researchers by reducing the time to obtain results and ensuring reliable data with total protein normalization. Using similar techniques, researchers also analyze the purity of purified proteins.

Analyze the Immune Response

Once a vaccine prototype is developed, immunological studies can begin. Whether in preclinical animal models or human samples during clinical trials, vaccine researchers must assess if their vaccine candidate induces a protective immune response with different doses, formulations, or adjuvant additions.

A hallmark of an active immune response is an increase in cytokine signaling and inflammation. The Bio-Plex Multiplex Immunoassay system and assay kits that quantify biological targets characterize the responses to infectious disease by measuring antibodies, pro-inflammatory cytokines, and chemokines from serum or other host samples.[3] The kits consist of premade panels or assays that can be customized for new targets.

A successful vaccine candidate also induces immune effector cell activation. Because individual responses to immunization are diverse, immunophenotyping is essential for informing vaccine development. Using flow cytometry instruments such as Bio-Rad's ZE5 Cell Analyzer, researchers evaluate vaccine candidates' abilities to stimulate various T cell responses and detect pro-inflammatory cytokines and their receptors in a high-throughput and multiplex manner.[4]

Verify Immunity

Researchers must ultimately verify that their candidate protects vaccinated individuals from infection. In preclinical studies, researchers quantitate the viral load in samples for vaccine challenge experiments. They first inject animals with precise amounts of virus to determine the dosages required to cause pathology. After vaccination, scientists administer their pathogen at the calculated dosages to confirm the vaccine's effectiveness. Similarly, in human clinical trials, viral dosage must be accurate to ensure quality results and the participants' safety. Finally, viral vector vaccines must be measured using similar methods to ensure that a safe dosage is applied. Quantitative PCR (qPCR) is a common tool for calculating the amount of virus (viral titer) in a sample. However, ddPCR technology is now the gold standard method for determining vector genome titers.[5] Experiments performed on ddPCR systems return more accurate data than qPCR and are easier to perform as they do not require a standard curve.[6]

Learn about Bio-Rad's solutions supporting COVID-19 vaccine development and testing.

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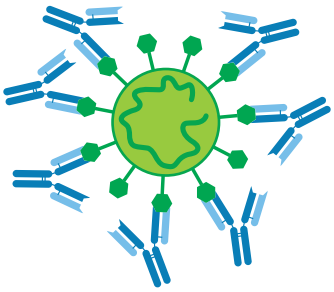
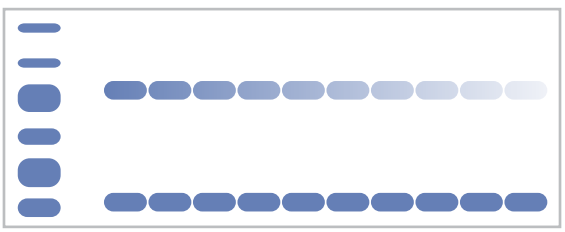
BUILDING A BETTER VACCINE

Throughout vaccine research and development, scientists must analyze their antigens, track the immune response, and determine viral loads. Modern instruments and assays aid vaccine developers and ensure that they receive fast and reliable results.



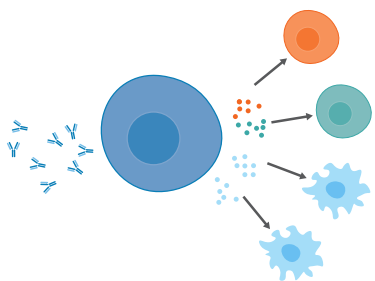
Identify an Antigen

- Find an antigen that induces protective immunity by assessing interactions with neutralizing antibodies using the ZE5 Cell Analyzer.
- Detect antigen variants with Droplet Digital PCR (ddPCR) using a QX200 or QX ONE instrument.



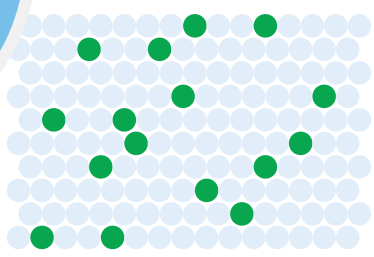
Verify Expression

- Develop a vaccine that delivers the candidate antigen or its sequence.
- Assess antigen production and purity in the chosen system by western blot and SDS-PAGE.



Analyze the Immune Response

- Track immune responses to immunization by measuring chemokines and cytokines with Bio-Plex Immunoassays.
- Phenotype immune cells that respond to vaccination with the ZE5 Cell Analyzer



Verify Immunity

- Determine the viral load required to induce pathogenicity with ddPCR systems.
- Quantitate viral titer for vaccine challenge experiments with ddPCR systems.

MAINTAINING QUALITY ON A LARGE SCALE

Vaccines must be manufactured in large quantities for clinical trials and subsequent distribution. To ensure a uniform, high-quality product where each lot meets safety and efficacy requirements, manufacturers must put their vaccines through numerous tests. Certain vaccine manufacturing and quality control processes, such as cell line development, impurity detection, and potency measurements, benefit from modern instruments and techniques that help vaccine manufacturers produce consistent products.

Cell Line Development

To react with the immune system as expected, vaccines must always deliver the same dose of identical antigen. For large-scale, protein-based vaccine production, vaccine developers create cell lines that express target antigens and remain stable over numerous passages. During initial cloning of the antigen's DNA into the cell line of choice, researchers find clones that express the antigen at high levels by sorting with flow cytometry. After flow cytometry identifies promising candidates, researchers can verify protein expression by western blot and assess genetic stability by measuring the transgene's copy number with Droplet Digital PCR (ddPCR). By partitioning amplification reactions over thousands of droplets, Bio-Rad's ddPCR technology precisely measures even minute quantities of target DNA and reliably detects small variations in copy number. These measurements highlight potential expression differences in a cell line over time,[1] which allows researchers to identify stable clones that produce large amounts of antigen.

Detecting Impurities

Once they purify target proteins using chromatography, scientists ensure that no other host cell protein (HCP) was collected by performing SDS-PAGE and gel staining with dyes such as Coomassie blue or SYPRO ruby.

Host cell DNA (HCD) is another notable impurity that researchers must track. During manufacturing, host cells may lyse and release their HCD. Additionally, integrated viral DNA segments may be freed by cell lysis. Because of these and concerns around vaccine efficacy, regulatory agencies require that researchers report HCD levels in biologics;[2] however, after extensive purification steps, HCD levels should be minute. ddPCR technology is sensitive enough to directly quantify extremely small amounts of HCD and provide the DNA size measurement of HCD without the need for DNA purification steps, to verify a vaccine product's purity.[3]

Assessing Potency

While the testing process is similar for many protein-based vaccines, viral vector and nucleic acid vaccines have special needs. Choosing the proper dosages of vaccines that deliver DNA or RNA into host cells requires additional measurements by ddPCR technology. Researchers must precisely measure the concentration of viral vectors and lipid nanoparticles. Once vaccines reach host cells, scientists use ddPCR technology to determine their infectivity by measuring the amount of target sequence delivered within host cells. Measuring the vaccine payload ensures that scientists administer an efficacious, non-toxic dose.

Certain vaccine manufacturing and quality control processes, such as cell line development, impurity detection, and potency measurements, benefit from modern instruments and techniques that help vaccine manufacturers produce consistent products.

Please see references on page 7

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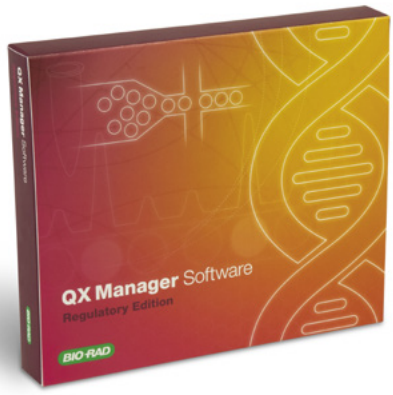

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