White Paper

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Quality Assurance in In Vitro Transcribed (IVT) RNA Vaccine Development using Agilent Automated Electrophoresis Instruments

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Abstract

Highly adaptable, efficient, and ideally suited for fast production, in vitro transcribed (IVT) RNA vaccines have emerged as a valuable tool against infectious diseases. The exceptionally successful IVT RNA vaccines against SARS-CoV-2 were built on years of research into mRNA. As infectious diseases rise in number and spread rapidly across the world, there is an urgent need for expeditious development and comprehensive distribution of vaccines against both known and previously unknown pathogens. Correspondingly, these vaccines drive a rising need for robust, reliable, rapid, and high-throughput quality control (QC) testing. The Agilent automated electrophoresis systems offer platforms for nucleic acid QC. Agilent instruments are used in QC during vaccine production and are ideally suited for in-process quality checks, and purity and integrity testing of the final vaccine.

Introduction

Pandemics

Pandemics such as COVID-19 are not new phenomena: since the first agricultural revolution 12,000 years ago during which humans settled into villages to cultivate crops and domesticate animals, societies became more sedentary and infectious diseases spread rapidly[1]. Among the most lethal pandemics are the Justinian Plague (541 AD), which was responsible for at least 30 million deaths, and the Black Death (1347—1351 AD), which killed approximately 50 million people[1,2]. About a century ago, the 1918-1919 influenza pandemic, known as the Spanish Flu, took a deadly toll[1]. Still today, pandemics continue to present a risk. In the past two decades, several pandemics emerged: H1N1 "swine" influenza (2009), chikungunya (2014), and Zika (2015), as well as pandemic-like emergences of Ebola fever over large parts of Africa (2014 to present)[1]. The timing and fatalities of these and other pandemics is shown in Figure 1.

Zoonotic diseases

About 60 percent of human infections are estimated to have an animal origin, including some with deadly consequences such as influenza, smallpox, measles, and bubonic/pneumonic plague[3]. One such infection, COVID-19, is caused by the novel coronavirus SARS-CoV-2. A variety of coronaviruses exist, which cause illnesses ranging from the common cold to more severe acute respiratory diseases. Of all new and emerging human infectious diseases, some 75 percent "jump species" from animals to people[4].

History of vaccines, vaccine development

As early as 1000 AD, cowpox inoculations were performed in China to create immunity against smallpox. It was over 800 years after the first smallpox vaccine was used before the next vaccines, against rabies and cholera, were developed[5]. Additional vaccines were developed through the 1930s against many diseases, including diphtheria, tetanus, plague, and tuberculosis. In the mid-twentieth century, research and discovery led to the development of several vaccines against common childhood diseases such as measles, mumps, and rubella[5]. The use of vaccines has led to the successful eradication of diseases such as smallpox. While the earliest vaccines used trial-and-error approaches, current vaccines are based on increasing knowledge of microbiology, virology, mechanisms of infection and immunity, and the biology of the infecting organisms, including basic biochemical structures and genetic sequences[5].

As the possibility of new diseases continue to arise and with worldwide spread of severe infections, which can occur rapidly, there is the potential urgent need for expeditious development and comprehensive distribution of new vaccines[6]. Recent advances in vaccine development, leading to the highly successful COVID-19 vaccines, were made possible by decades of research into mRNA, including its properties and immunogenicity. This research could easily be adapted to other pathogens, including those yet unknown, expanding future vaccine production.

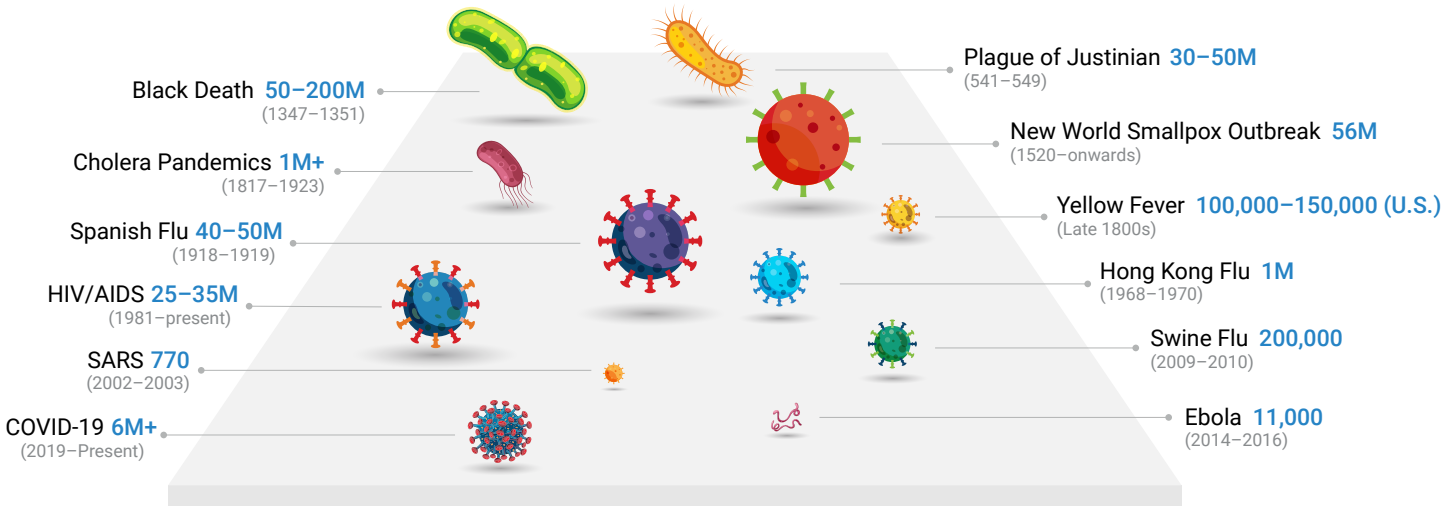

Figure 1. Infectious disease outbreaks over time. The approximate numbers of deaths are given (blue) along with the time of each pandemic. The COVID-19 pandemic is ongoing and has claimed over 6 million lives worldwide as of January 2022. Adapted from LePan, N. Visualizing the History of Pandemics. Visual Capitalist. 2020 Infographic: The History of Pandemics, by Death Toll (visualcapitalist.com).

mRNA: key discoveries

Research into RNA has been ongoing for decades. Notably, the development of synthetic genes led to the exploration of in vitro transcribed (IVT) RNA in therapeutic applications, including vaccination approaches for infectious disease. However, the therapeutic use of IVT RNA has been hampered by two major hurdles. First, IVT RNA activates the host immune response which can destroy foreign RNA prior to translation. Second, the large size and negative charge of mRNA impedes diffusion of mRNA across cell membranes. The research into mRNA over the past two decades has helped overcome these challenges and laid the foundation for IVT RNA therapies[7] (Figure 2).

Two pioneering scientists in the advancement of mRNA vaccine technology are Dr. Katalin Karikó and Dr. Drew Weissman. Karikó et al. found that mRNAs containing pseudouridine (Ψ), a naturally occurring modified nucleoside, were translated more efficiently and showed less activation of the host immune response[8, 9]. The authors, therefore, suggested that modification within the foreign RNA might allow the RNA to avoid host immune activation. Additionally, the large size and negative charge of mRNA makes it difficult to deliver mRNA into a cell, necessitating the use of a vehicle to transport IVT RNA. Years of research into the use of lipid nanoparticles (LNPs) as mRNA carriers led to their use as efficient and safe delivery vehicles for IVT RNA vaccines[10, 11, 12]. Today, modified RNA and LNPs such as these are being used for the Pfizer-BioNTech and Moderna COVID-19 vaccines[13].

Translational and clinical research on mRNA-based vaccines progressed steadily, from animal models in the 1990s onwards, to the first human cancer immunotherapy trial using direct injection of mRNA in 2009. The first successful demonstration of an infectious disease vaccine in humans was with a rabies vaccine in a clinical trial begun in 2013[14]. These and other key advancements in mRNA research are shown in Figure 2.

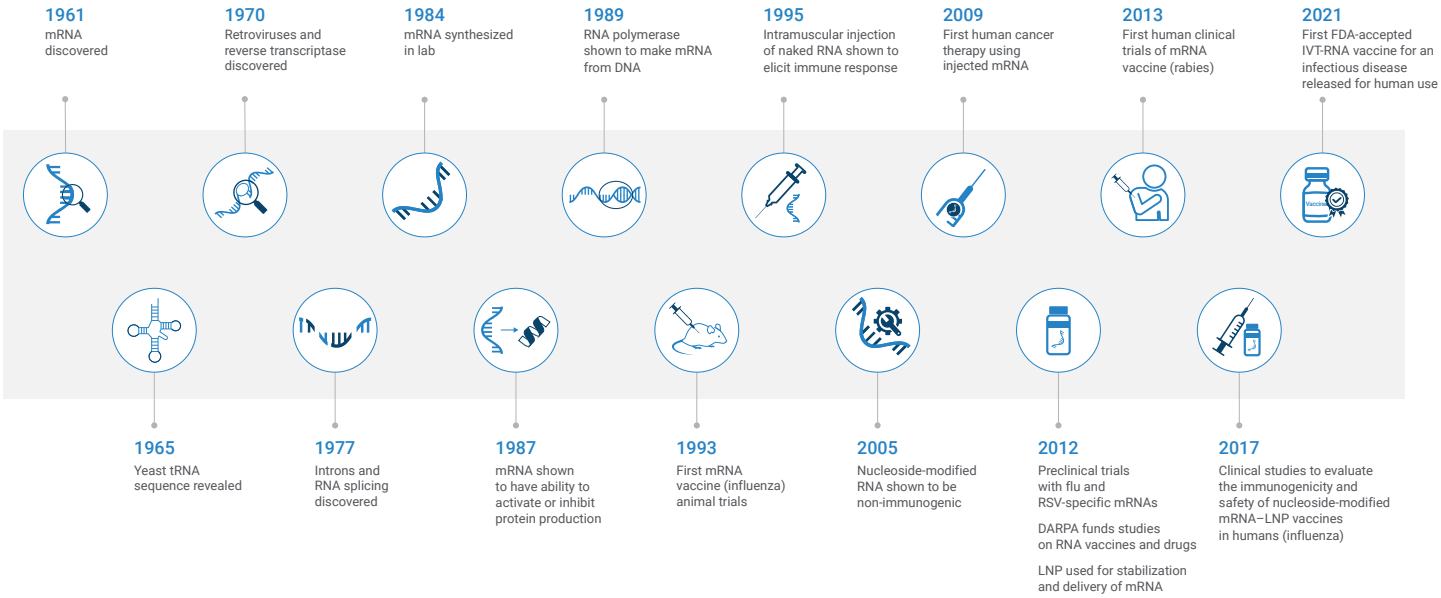

Figure 2. Important RNA research milestones. Timeline of some key achievements in RNA research which contributed to the development of IVT RNA vaccines against COVID-19.

Collectively, these findings laid the foundation for the design of therapeutic mRNAs. Currently, there are almost 2,000 clinical trials, worldwide, involving the use of therapeutic mRNAs[15]. These trials range from protein replacement therapies to vaccines against various viruses including SARS-CoV-2[15]. Figure 3 shows other potential therapeutic applications of IVT RNA, including cancer immunotherapies and infectious disease vaccines.

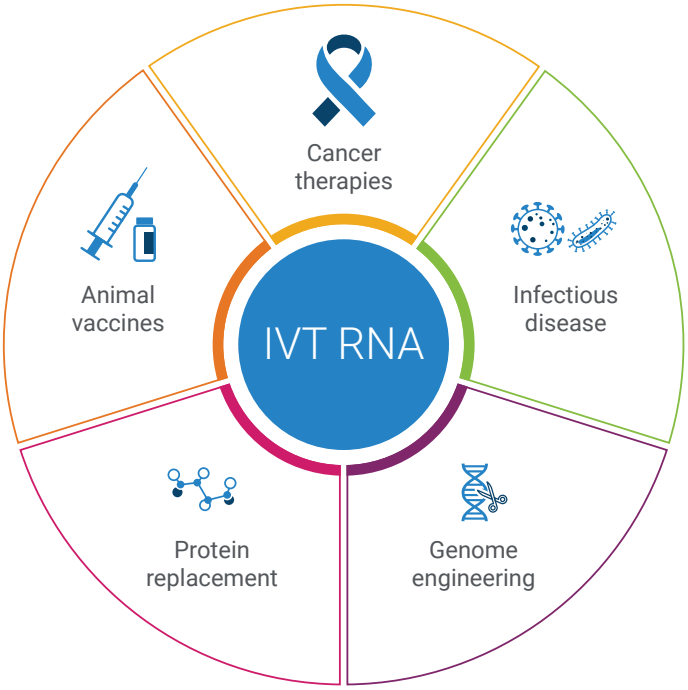

Figure 3. Potential therapeutic applications of IVT RNA. IVT RNA therapies involve introducing genetic material like mRNA, which is a template for a particular protein, into a cell where it can prevent or alter a disease[7]. Some potential therapeutic applications of IVT RNA are depicted.

Vaccine development: conventional vaccines vs. COVID-19 mRNA vaccines

Vaccine development is traditionally a complex and time-consuming process that typically takes 10 to 12 years[16]. As illustrated in Figure 4, vaccine development usually begins with an exploratory stage focusing on basic research and computational modeling to identify potential natural or synthetic antigens as vaccine candidates. Preclinical studies generally take about 18 to 30 months, and human clinical trials can take several years. After completion of these trials, the vaccine safety and efficacy data are reviewed for approval by regulatory bodies, such as the Food and Drug Administration (FDA) in the US or the European Medicines Agency in the EU[17].

In COVID-19 vaccine development, the 10 to 12-year timeline was significantly reduced to 12 to 24 months[16] (Figure 4). A large portion of this reduction in time was made possible by decades of research into RNA and using the data from the preclinical development of vaccine candidates for SARS-CoV-1 and MERS-CoV, thus omitting the initial exploratory phase[16]. Additionally, overlapping clinical trial phases and Emergency Use Authorization (EUA) contributed to the rapid development of the COVID-19 vaccine[16].

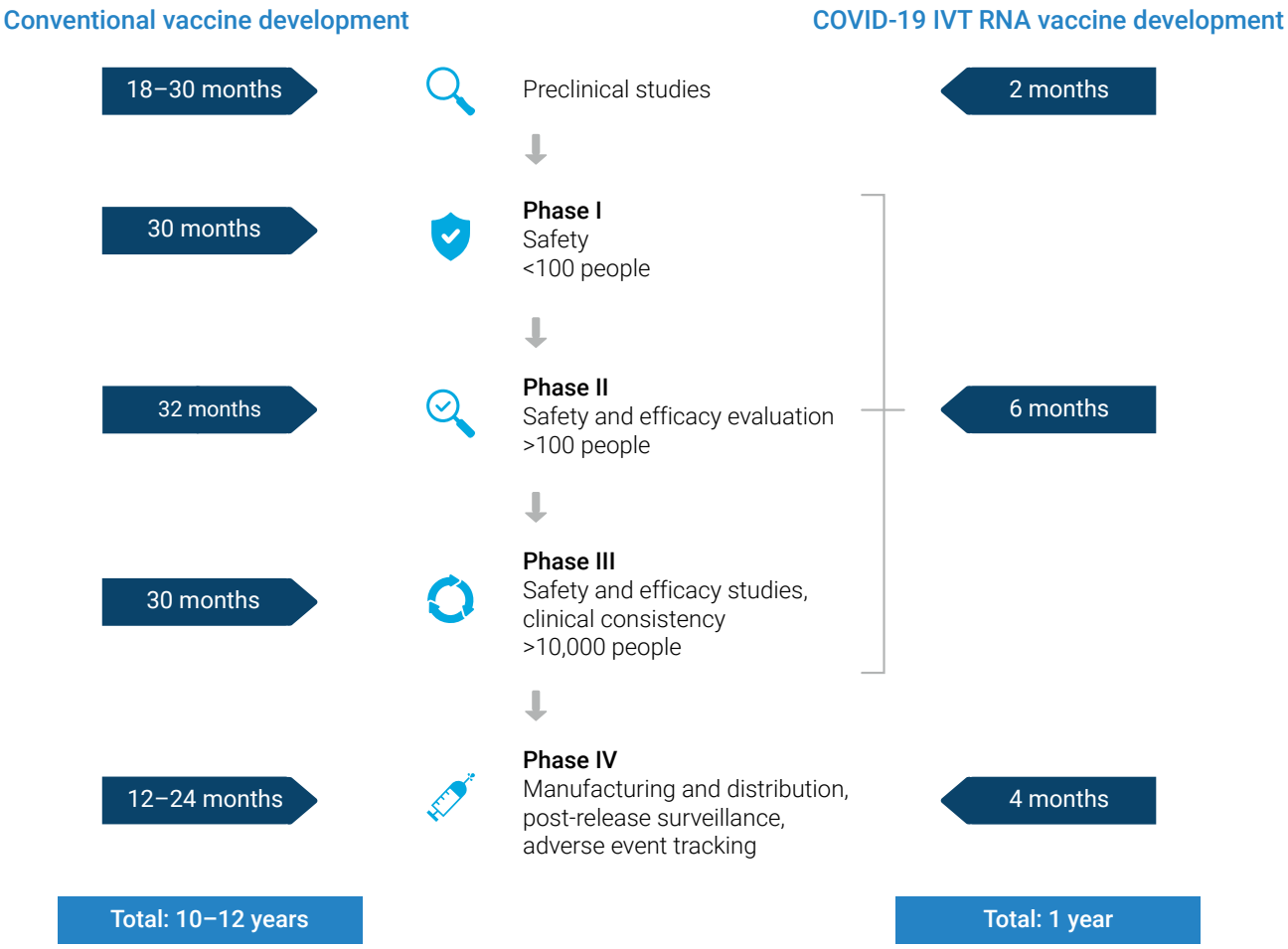

Figure 4. Timeline of traditional and COVID-19 IVT RNA vaccine development. Conventional vaccine development takes 10-12 years (left) compared to the COVID-19 IVT RNA vaccine, which was developed in one year (right). Data obtained from decades of research into RNA, and the preclinical development of vaccines against SARS-CoV-1 and MERS-CoV, along with overlapping clinical trials and Emergency Use Authorization (EUA) were key in the substantially reduced time needed to develop the COVID-19 IVT RNA vaccines.

mRNA vaccines and COVID-19

When the deadly SARS-CoV-2 virus emerged in late 2019 and the pandemic spread rapidly around the world, the culmination of decades-long research into mRNA led to the rapid development of the highly successful COVID-19 mRNA vaccines[17]. The development of these vaccines started once the viral genome sequence was available. A mere two months after sequence identification of SARS-CoV-2, Moderna started clinical testing of its novel mRNA-based vaccine mRNA-1273[18]. On 11 December 2020, less than a year after SARS-CoV-2 was identified, the first vaccine consisting of mRNA encoding the spike protein of the virus was granted EUA by the US FDA: BNT162b2, developed by Pfizer-BioNTech[19]. A week later, Moderna received an EUA for its vaccine[20].

As of January 2022, 59.6% of the world population has received at least one dose of a COVID-19 vaccine, including, viral vector, inactivated virus, live attenuated virus, DNA, and mRNA vaccines [7, 21]. Over 11 billion doses of the COVID-19 vaccines were distributed in 2021, approximately 27% of these being mRNA vaccines[22, 23]. There are still over three billion individuals worldwide yet to receive a single vaccine dose[22]. Additionally, most fully vaccinated individuals have yet to receive a booster shot[22]. It is likely that more vaccine will need to be produced in 2022 than was produced in 2021.

Vaccine production

The steps to produce IVT RNA used in mRNA vaccines are well established: the target pathogen is identified and sequenced, candidate antigen sequences are designed, and plasmid DNA vectors prepared. This is followed by synthesis of the target RNA from DNA templates, generated by linearization of the purified plasmid. This established procedure, shown in Figure 5, is easily adapted to novel sequences. For example, both Pfizer-BioNTech and Moderna are expecting to release Omicron-specific vaccines in March of 2022 [25, 26].

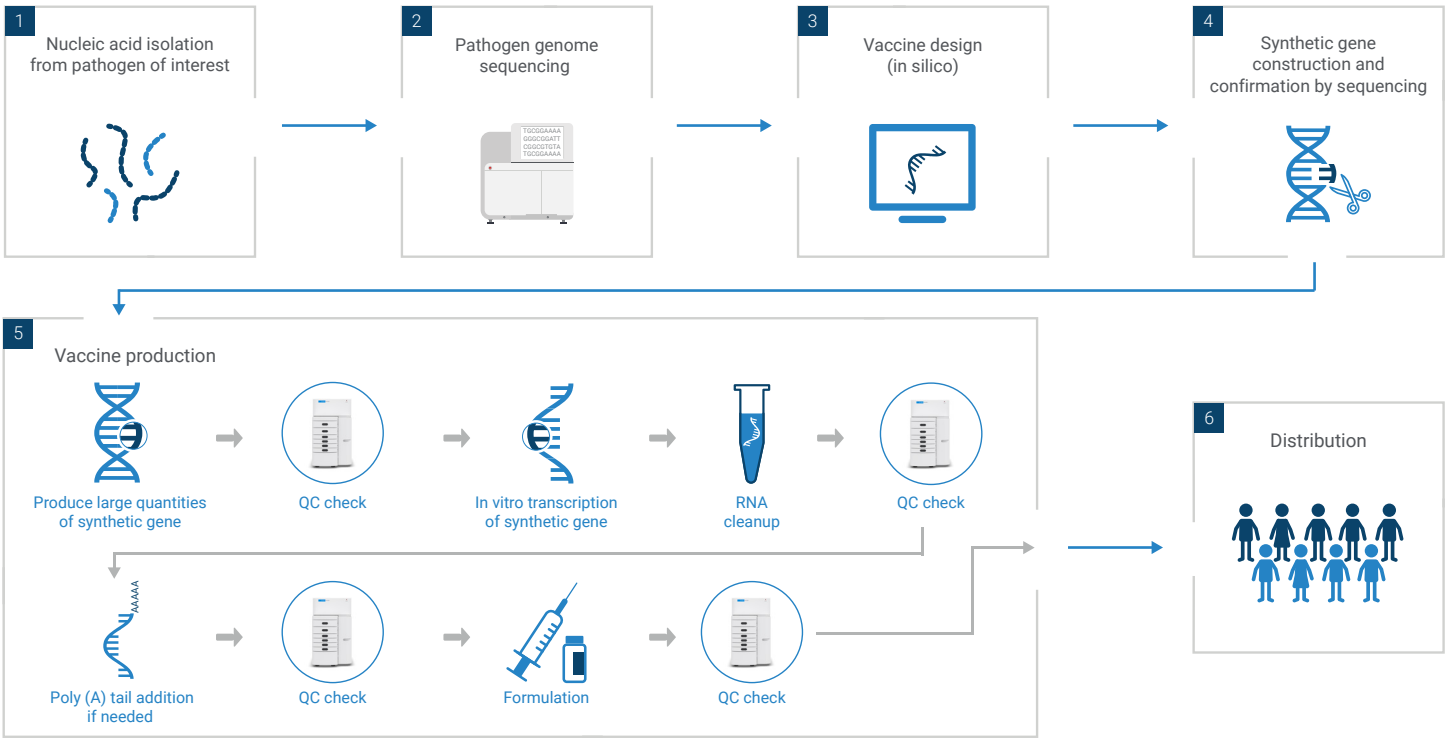

Figure 5. Schematic of IVT RNA vaccine development. The steps of COVID-19 IVT RNA vaccine development are shown, from the initial isolation of the pathogen to vaccine distribution. Included in this workflow are potential QC steps where the Agilent Fragment Analyzer and Agilent TapeStation systems can be used to help ensure the quality of the sample before moving to the next step, and helping to ensure that the final vaccine is suitable for distribution.

For the manufacture and control of mRNA vaccines using good manufacturing practices (GMP), the World Health Organization (WHO) requires the establishment of quality control systems[27]. They state "…adequate control of the starting raw materials and manufacturing process is as important as that of the final product. Regulatory considerations therefore place considerable emphasis on the control strategy of the manufacturing process of the vaccine as well as on comprehensive characterization and release testing of the bulk substance and the vaccine itself."[27]

The WHO further recommends that throughout the process, several in-process control tests should be established to allow quality to be monitored for each batch or lot from the beginning to the end of production. The purity of final RNA is especially vital for the potency of mRNA vaccines[24]. During IVT RNA vaccine production, typical analyses of the linearized plasmid, as well as of the final product address identity, appearance, content, integrity, residual DNA, endotoxin contamination, and sterility[8]. Figure 5 shows the in-process QC steps, which include quality and size of the linearized plasmid, purity of the IVT product, quality and size following addition of the

poly(A) tail, and the purity of the final product. The Agilent automated electrophoresis instruments are ideally suited for several in-process quality checks as well as for testing the vaccine in its final form, as evidenced by their use in quality control by the manufacturers of the COVID-19 mRNA vaccines.

While the highly successful SARS-CoV-2 vaccines are currently the most prominent example of mRNA vaccines, several other applications are being actively investigated, and therapeutic uses of mRNA will continue to proliferate. Over 200 mRNA vaccines, including several for influenza, are in or are soon to enter clinical trials[15]. Thus, mRNA vaccine technology shows great promise in meeting the challenge for rapid development and large-scale production of new vaccines. With the increasing number of vaccine targets, coupled with high production goals, there is a rising need for robust, reliable, rapid, and high-throughput quality control testing during vaccine production.

Agilent automated electrophoresis systems in mRNA vaccine production

The Agilent automated electrophoresis instruments, including the Bioanalyzer, Fragment Analyzer, and TapeStation systems, have played an important role in quality assurance of the SARS-CoV-2 vaccines currently being administered. The instruments are each capable of quantitative and qualitative analysis of DNA and RNA with a broad reagent portfolio, ideal for many applications.

While the details of vaccine manufacturing are kept highly confidential by companies, Pfizer revealed some key aspects of its COVID-19 vaccine manufacturing process to USA Today. The article states, "More than half of the production time for Pfizer's COVID-19 vaccine is devoted to testing and quality assurance – making sure the resulting product, at each stage is safe, pure and exactly the same as the tested vaccine that proved effective."[28] The entire vaccine production process is split among three sites for maximal efficiency: purification of the plasmid which encodes the mRNA of the spike protein at the first site, production and purification of mRNA at the second, and finally, encapsulation of mRNA into lipid nanoparticles, followed by distribution into vials at the third. Figure 6 summarizes the reported timeline and depicts where the Agilent Fragment Analyzer can be used at different steps in the Pfizer vaccine manufacturing process, to assure purity and integrity of the intermediates, as well as the final product.

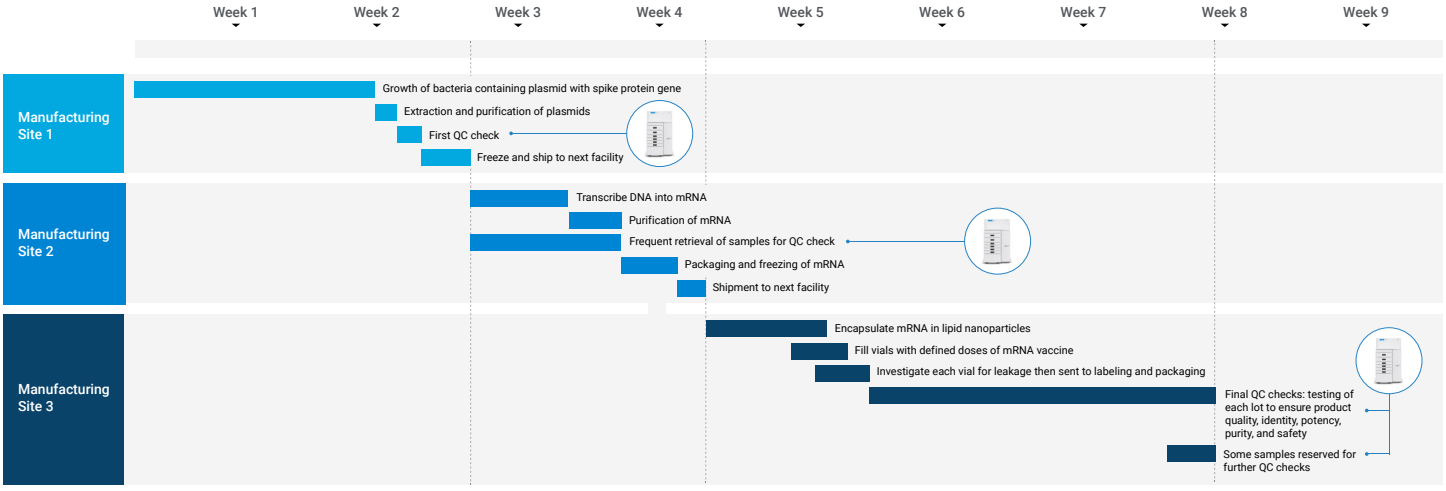

Figure 6. Steps in Pfizer-BioNTech COVID-19 vaccine production, QC steps, and role for Agilent Technologies. The production of the Pfizer-BioNTech COVID-19 vaccine, split between three different production sites, takes just over eight weeks. Among the many different QC steps, the Agilent automated electrophoresis systems can be used to ensure the size and quality of the samples at various steps in the workflow, as indicated by the inset images of the Agilent Fragment Analyzer system. By using the Fragment Analyzer at each site, quality can be ensured across the manufacturing locations. Timeline adapted from Weise and Weintraub[28].

Some recent published examples which use Agilent instruments for quality control during vaccine production are discussed below.

Example 1: Pfizer–BioNTech BNT162b vaccine production, and quality assessment using the Agilent Fragment Analyzer[13]

The preclinical development of two Pfizer-BioNTech mRNA vaccine candidates (BNT162b1 and BNT162b2) encoding immunogens derived from the spike glycoprotein (S) of SARS-CoV-2, formulated in lipid nanoparticles, was reported in September 2020[13]. While both candidate vaccines induced strong antigen-specific immune responses in mice and macaques, BNT162b2 was selected over BNT162b1 for further clinical testing due to its greater tolerability with comparable immunogenicity. RNA integrity was assessed by capillary electrophoresis, with the Fragment Analyzer.

Example 2: Assessment of stability of the Moderna and Pfizer–BioNTech BNT162b vaccines using the Agilent Bioanalyzer[29]

Once vaccines were available, there was an urgent need for vaccine deployment to sites around the world without compromising the integrity of the vaccines. Initially it was thought that reconstituted vaccines would not be stable. Grau et. al. demonstrated that both the Pfizer-BioNTech and Moderna vaccines retain their integrity at ambient temperature under movement conditions consistent with agitation that would occur during three hours of driving on roads with good condition. This implied that vaccines could be more widely distributed after reconstitution, thus improving the efficiency of vaccine distribution, particularly via ground transportation in rural areas[29]. While the Bioanalyzer was used to analyze mRNA integrity, the Fragment Analyzer and the TapeStation are also well-suited for such analysis and could handle a higher throughput of samples.

Example 3. Quality assessment of a self-replicating RNA vaccine against SARS-CoV-2 using the Agilent Fragment Analyzer[30]

A self-replicating RNA vaccine was developed by Arcturus Therapeutics with the goal of a single low-dose administration, using proprietary self-transcribing and replicating RNA (STARR technology) against SARS-CoV-2[30]. In assessing the immunogenicity and host response in a mouse model, the authors found that self-replication amplified the immunogenicity of the RNA vaccine, showing potential for an effective single-shot vaccination against COVID-19. Quality and integrity of the purified RNA, as well as the final vaccine lipid nanoparticle, was assessed using the Fragment Analyzer.

Example 4: Quality assurance of neoantigen-encoding messenger RNA manufactured under GMP for early-phase cancer vaccine clinical trials using the Agilent Fragment Analyzer[31]

Neoantigens are mutated peptides expressed in a tumor, which are rarely shared between patients. Hence, including these antigens in a vaccine requires the production of individual batches of patient-tailored mRNA. A dendritic cell vaccine targeting tumor neoantigens was developed for evaluation in lung cancer patients, and in their GMP facility, the authors demonstrated that the process delivers consistently high-quality patient-tailored neoantigen mRNA[31]. mRNA identity and integrity were analyzed by capillary gel electrophoresis (CGE) using the Fragment Analyzer. The authors also established the storage stability of the neoantigen mRNA by analyzing the integrity of the mRNA using CGE. The quality assessment approach they described was approved by the competent regulatory authority in Belgium (Federal Agency for Medicines and Health Products) as part of the investigational medicinal product dossier of their vaccine candidate.

Agilent automated electrophoresis systems

The Agilent Fragment Analyzer systems and the Agilent TapeStation systems, shown in Figure 7, enable nucleic acid analysis with flexible throughput options. Both the Fragment Analyzer and the TapeStation require a minimal amount of sample (1-2 µL). The Fragment Analyzer systems offer reliable DNA and RNA QC analyses, including accurate sizing of IVT RNA through 9,000 nt, to confirm transcription efficiency and perform smear analysis to assess minute amounts of degradation[32, 33]. The systems are available in a range of throughputs, holding up to three 96-well plates, and their benefits include unattended operation, reduced sample handling, and decreased preparation time[34]. The TapeStation systems, based on ScreenTape technology, offer ease-of use in combination with a fast analysis time of 1-2 minutes per RNA and DNA samples. Ready-to-use consumables ensure straightforward operation with minimal hands-on time[35].

Figure 7. Automated Electrophoresis Solutions for IVT RNA QC. The Agilent Fragment Analyzer systems and the Agilent TapeStation systems offer reliable nucleic acid quality control for a variety of applications, including accurate sizing of IVT RNA.

Conclusion

Vaccines produced using IVT RNA technology are highly adaptable, efficiently made, and ideally suited for fast production. IVT RNA vaccines are a valuable tool against COVID-19 and are in trials for other applications, including vaccines for various infectious diseases and cancer immunotherapy. The Agilent automated electrophoresis systems are well-suited to serve QC needs in current production markets, and are primed to play a continuing vital role in assuring in-process quality as well as quality of the final product. As the number of vaccine targets and production goals increase, so does the need for robust, reliable, and rapid quality control methods.

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