Stability Studies of Vaccines

12

Ajab Khan, Anam Ahsan, Muhammad Asim Farooq, Mirza Muhammad Faran Ashraf Baig, and Qurat-ul-ain Aslam

Abstract

Edward Jenner laid the foundation of vaccination as he was the first to introduce and use the procedure as well as pursued his scientific investigation against the deadly smallpox. Due to his work and the recent advancements in science, now the world claims that smallpox has been eradicated from the world. For long-lasting effective immunization, both the humoral- and cell-mediated parts of the adaptive immunity need to be stimulated. There are almost seven types of vaccines that are currently in use or in the stage of development. The benefit-risk profile (efficacy) of each vaccine is constantly evaluated during the entire process right from the individual component pre-clinical evaluation (in vitro and in vivo) and clinical (human trial) and developmental phase till the end use. The Vaccine Adverse Event Reporting System (VAERS) used by the Centers for Disease Control and Prevention (CDC) and Food and Drug Administration

A. Khan (✉)
Department of Biochemistry and Molecular Biology, Shanxi Medical University, Taiyuan, Shanxi, People's Republic of China
e-mail: drajab22@gmail.com

A. Ahsan
College of Veterinary Medicine, Shanxi Agricultural University, Jinzhong, China

M. A. Farooq
Department of Pharmaceutics, School of Pharmacy, Pharmaceutical University, Nanjing, Jiangsu, People's Republic of China

M. M. F. A. Baig
State Key Laboratory of Analytical Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing, China

Q.u.-a. Aslam
Drug Testing Laboratory, Faisalabad, Pakistan

M. S. H. Akash, K. Rehman (eds.), *Drug Stability and Chemical Kinetics*,
https://doi.org/10.1007/978-981-15-6426-0_12

(FDA) monitors post-licensure vaccine efficacy and safety using various methods and techniques. Advanced kinetic models are used to calculate the degradation kinetics rate of biological products such as protein and virus-based vaccines and also an emulsion-based adjuvant vaccine. Statistical tools are used to select an optimal number based on the variable parameters used for fitting of experimental data obtained from different steps of kinetic models.

Keywords

Vaccination · Smallpox · Humoral · Cell mediated · Kinetic models · Centers for Disease Control and Prevention

12.1 Introduction

For decades, smallpox had threatened the globe by causing countless deaths, for which the world is thankful to Edward Jenner (Fig. 12.1), whose innovative and remarkable contribution to immunization ultimately eradicated smallpox and also laid a foundation for the vaccine's development. Although China and India have used the principles of vaccination since thousands of years by inoculating live and

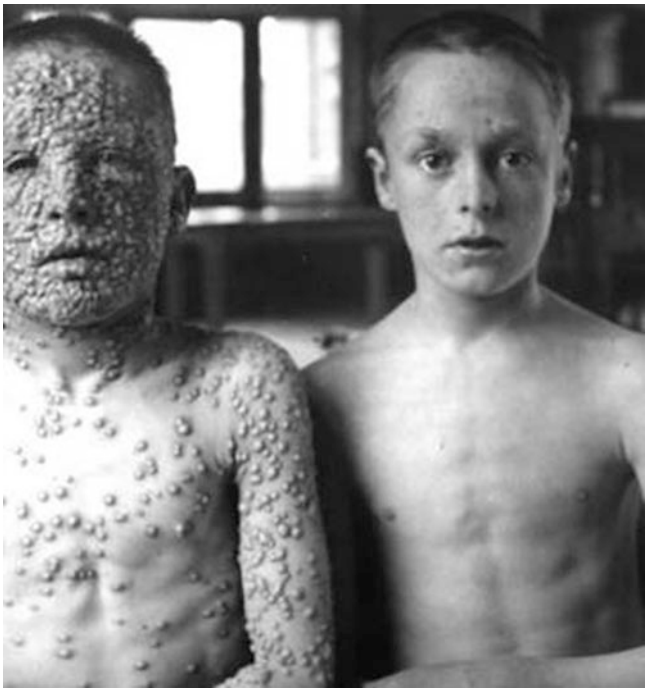

Fig. 12.1 Photo of the same boy prior and post smallpox infection. This is the reason why this disease was called variola (means stain or mark on the skin), speckled monster in the eighteenth century, and small pockes in the fifteenth century

virulent smallpox virus for later protection. However, Edward Jenner is honored as a pioneer of vaccine development almost 200 years ago, who gave it the name of *vacca* in 1798, which means cow [1]. From this first-ever development till the advancement of novel vaccines, vaccination has overwhelmed the consequences of catastrophic infections. Briefly, in the eighteenth century, the world had the vaccinia virus vaccine, and Louis Pasteur and Emile Roux in the nineteenth century explained that attenuated or inactivated organisms provide prophylaxis, and finally an accelerated advancement was observed to produce immunization using advanced technologies [2]. Today's research is focused to develop novel vaccines to defeat and eradicate deadly infectious diseases by boosting and engineering the immunity [3].

Vaccines are actually biological accumulation of antigens which are designed to provoke adaptive immunity by mimicking an infection to produce antibody and memory cells without causing illness [4]. There are different stages to measure vaccine's efficacy which starts from pre-licensure stage. A randomized controlled trial is carried out in which the disease incidence rate between non-immunized and immunized volunteers is considered as a direct effect of the vaccine [5, 6]. Estimation of vaccine effectiveness is usually measured from studies carried out post-licensure, which showed the actual protection against disease under real-life condition [5]. Both efficacy and effectiveness of the vaccines are calculated using the following formula:

$$VE = R_{\text{unvaccinated}} - R_{\text{vaccinated}} \div R_{\text{vaccinated}} \quad (R = \text{Risk or Rate})$$

Keeping in view this background, we will first shortly highlight the immune system and its types, short history of vaccine development, and types of vaccines to build a baseline study and then will focus on the efficacy and stability of vaccines.

12.2 Immune System

We are living in the world surrounded by an ocean of microorganisms called pathogens, both harmful and harmless to our body. These microorganisms in one way or the other get access to the body, select a predilection site, start multiplication, and thus harmfully affect the body. Nature has provided a protective screening system to the body, the main function of which is to screen these organisms at various stages and not allow them to enter into the body. In case they enter into body, the body has a defensive mechanism which first labels them as a foreign enemy and alerts the body's defense system to start a combat against them.

12.2.1 Types of Immune Systems

This body's defense system, which is named as immune system because of the immune cells involved in this process, can be divided into two main types: innate/general and adaptive/specific immune systems. Both of these types communicate

with each other to provide effective immunity to the body against the pathogens which gain access to the body.

12.2.2 Innate or General Immune System

The innate immune system is the first line of defense which provides the first-ever barrier against the pathogens trying to invade the body. This system provides a variety of protective measures which are continuously in function and are not specific to a particular pathogen; that's why it is termed as general immune system. Due to specificity of innate immune cells toward conserved molecular patterns, which are found only on microorganisms and not on the host cells, which prevents host cells from attack. This system lacks memory cells, so repeated exposure of the same pathogen limits the improvement of this system. Anatomical barriers (intact skin), mucous membranes, and acidic environment (pH 3–5) of the skin prevent the entrance and growth of pathogens. Mucus and cilia of the mucous membranes trap the microorganisms and propel them out of the body [7, 8].

Furthermore, physiological barriers such as the normal temperature of the body, development of fever, gastric acidity, lysozyme (hydrolytic enzymes found in body fluids), interferon (produced by virally infected cells), and collectins (surfactant proteins present in serum, lung secretion, and mucus) are also parts of the innate immune system, which can inhibit, eliminate, and cleave directly (disrupting lipid membrane) or indirectly by clumping the pathogens to enhance their susceptibility to phagocytosis [7, 9].

The complement system is divided into three pathways based on the way they are activated. Classical, alternative or properdin, and lectin pathways are activated when IgM/IgG antibody binds to antigen, when C3b complement protein is deposited onto the microbial surfaces, and when plasma mannose-binding lectin (MBL) attaches to microbes, respectively. These pathways merge into a single pathway which leads to the formation of membrane attack complex that forms pores in the membrane of pathogens. The complement system is also involved in the opsonization of a particular pathogen to direct it into phagocytosis and trigger inflammation. The inflammatory response with five cardinal signs toward an injury or pathogen is also a part of the innate immune system [10, 11]. Pattern recognition receptors (PRRs) such as pulmonary surfactant protein, C-reactive protein, Toll-like receptors (TLRs), mannan-binding lectin (MBL), C-type lectin receptors (CLRs), MYC-associated factor X (Max), and neucleotide-binding oligomerization domain (NOD) are membrane proteins found on all innate immune system cell's membrane. The PRRs recognize pathogen-associated molecular patterns (PAMPs) including LPS, peptidoglycans, lipoproteins, and flagellin and trigger cytokine release, opsonization, complement activation, and phagocytosis. PAMPs are present only on microbial cells and not on human cells [10, 12, 13].

Phagocytic and granulocytic cells are the final soldiers of innate immune system which links it with the adaptive immune system. Monocytes circulating in the blood and macrophages present in the tissues are the main cells of this mononuclear phagocytic system whose main function is antigen presentation, cytokine production, and phagocytosis [14].

12.2.3 Adaptive/Specific Immune System

The adaptive immune system has the ability to adapt itself when exposed and is also specific against a particular pathogen with memory for repeated exposure [10]. Humoral immunity composed of B cells and cell-mediated immunity composed of T cells are two parts of adaptive immune system [15]. B cells are produced in the bone marrow, processed, matured, and also exposed to the extracellular pathogen/toxins in the lymph nodes. B cells recognize pathogens with lipopolysaccharide, dextran, and bacterial polymeric flagellin (without being processed by an antigen-presenting cells) and become activated which is a weaker response. The response of B cells which are activated by T helper cells is much better with effective memory and is used for long-term immunization. The activated B cells are converted into plasma cells which then begin to produce specific antibodies. IgM is the first antibody produced followed by the antigen-specific IgG antibody. IgD, IgA, and IgE are also produced, but IgG is the most important antibody related to vaccines [1, 16].

Cell-mediated immunity comprises of T cells, which are mature in the thymus and are activated against intracellular pathogens. CD4 or T helper cells and CD8 or T cytotoxic cells are the two main types of T cells. T helper cells recognize MHC II (major histocompatibility complex), which is present in all immune cells, and therefore are called the markers of the immune cells. T helper cells are further divided into Th1 responsible for cell-mediated immunity and Th2 responsible for antibody-mediated immunity. T cytotoxic cells, which are responsible for cell-mediated immunity, can recognize MHC I protein, which is present on the body's nucleated cells without RBCs and thus are termed as markers for the body cells. Compared with B cells, T cells can only recognize the antigens which are being processed by antigen-presenting cells [1, 16].

12.2.4 Immunization and Its Types

It is a process by which the immune system of an individual is armed against the harmful enemy called immunogen. Broadly it can be divided into passive immunization by transfer of preformed antibody and active immunization by exposure of the body to pathogenic agents. Passive immunization is for a time being and is finished when the inducted antibodies are destroyed. In neonatal life, maternal antibodies that are transferred through placenta and to the newborn through colostrum and milk are natural ways of passive immunity. Gamma globulins and antivenin are examples of artificial way of passive immunization. Active immunization is when an individual is directly exposed to pathogen and the body starts developing a long-term immunity compared with a passive immunity. Active immunization can also be developed naturally (exposure to influenza) and artificially (different types of vaccines used). A viral antigen that bounds with MHC I protein and presented at CD8 will initiate cell-mediated immunity, while a bacterial or parasitic antigen will be bound on MHC II molecules and presented on CD4, which will trigger antibody-mediated immunity

[17, 18]. A list of the different kinds of vaccines which are currently in use or in the stage of development is mentioned in Table 12.1.

12.2.5 History of Smallpox and Vaccine Development

Smallpox is a viral disease believed to have appeared and destroyed millions of human populations in the early eighteenth century throughout the world. It was named as speckled monster in eighteenth century in England, variola (means stain or mark on the skin) in Switzerland in 570 AD, and small pockes in the fifteenth century. It has a unique history as it was the first disease for which vaccine was developed and which was also eradicated from the world [1, 19]. The survivors of smallpox were immune to the diseases, and many efforts were performed to cure the diseases and decrease the devastating effects as much as possible. Earlier, inoculation, derived from a Latin word *inoculare*, which means "to graft," was considered an efficient way to treat smallpox. In this procedure, a smallpox virus (a wet lancet with fresh smallpox matter) was subcutaneously inoculated into a non-immunized individual. Later on, this procedure was named as variolation, which became very popular and decreased the fatality rate up to 10 times [8, 20]. In 1756, a boy named Edward Jenner was variolated against smallpox who developed mild case of smallpox with subsequent development of immunity and survival. Edward had a strong interest toward science and nature during his school life, and at the age of 13, he heard about a dairyman saying that "I shall never have smallpox for I have had cowpox, I shall never have an ugly pockmarked face." In that time, it was a very common belief that the dairymaids are somehow protected from smallpox. In 1796 Edward Jenner concluded that cowpox gives protection against smallpox and could be transmitted with unknown mechanism from one individual to another. From a cowpox-infected person's lesions, Jenner inoculated the boy, and after subsequent mild signs, the boy recovered on the tenth day after inoculation. The boy was inoculated again from a fresh cowpox vesicle, but this time the boy did not develop any signs of the disease. In 1798, he published a booklet which was based on his experiments on variolation. The conclusion of this publication was that the origin of cowpox is from horses, which is then transmitted to cows, and cowpox infection provides protection against smallpox. He called this procedure vaccination and the fresh cowpox material as vaccine, which was derived from Latin words *vacca* for cow and *vaccinia* for cowpox. The vaccination replaced variolation and became popular in the early eighteenth century throughout the world [1, 21].

12.3 Quality Control and Vaccine Development

The achievements and benefits of vaccines to the world can be measured from various factors, including eradication of smallpox in 1977; polio, which was eradicated very soon; and reduction of deadly outbreaks and consequent mortalities. Several vaccines approved for human use are listed in Table 12.2. For the

Table 12.1 List of vaccines currently in use or in the stage of development with advantages/disadvantages and examples

Name of vaccine	Procedure	Advantages	Disadvantages	Examples
Live attenuated	Laboratory weakened version of live pathogen	Produce both strong cellular and antibody response, produce long-term immunity, lifetime safeguard, single- or highly two-dose inoculation	Possibility of reversion to the virulent form, cannot be given to individuals with weak immunity (HIV or cancer patients)	Smallpox, type 2 poliovirus, bacillus Calmette-Guerin (BCG), measles-mumps-rubella (MMR) vaccine
Inactivated or killed whole-cell vaccines	Destruction of the pathogen by heat, chemicals (formalin), or radiation; therefore cannot replicate in the live organism	More stable and protective, do not require refrigeration and can be freeze-dried for transport, safer with fewer side effects	Produce weaker immune response due to lack of replication and fast clearance from the body, additional booster doses required to boost immune system for long-term immunity	Influenza, hepatitis A, Listeriosis
Subunit (protein-based subunit polysaccharide-based subunit conjugates)	Contains only the antigen or epitope (antigenic part) of the pathogen; 1–20 antigenic parts of microbes can be used instead of entire pathogen	Most readily stimulate the immune system, specific and late adverse reaction, can be used by every individual	Difficult procedure to know which antigen should be included, no confirmation regarding forming memory cells	Hepatitis Influenza *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae type b*
Toxoid (bacterial inactivated toxins)	Produced by inactivated (detoxified) bacterial toxins by formalin with ddH_2O or sometimes with heat or radiation	Produced immunity against bacterial toxins, cannot get back to its virulent form, cost-effective	Produced immunity against bacterial toxins	Diphtheria, tetanus, pertussis, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b (Hib)
Conjugate	Epitope linked to polysaccharides	To invoke an immune response against the weak antigens (specially bacteria)	Specially used in infants	*Haemophilus influenzae* type B (Hib), *Streptococcus pneumoniae*, *Neisseria meningitidis*

(continued)

Table 12.1 (continued)

Name of vaccine	Procedure	Advantages	Disadvantages	Examples
Naked DNA	Injected genetically engineered DNA in the form of plasmid DNA (pDNA) encoding antigen	Can be produced relatively easily at low costs and to produce both humoral and cellular immunities; pDNA is stable at room temperature which renders cold chain requirement	Has been approved for human use, and various vaccines are in clinical trials and some are approved for veterinary use	Cancer and HIV-AIDS (under trial)

development of a novel vaccine, sufficient and exact knowledge of its protective mechanism, i.e., to provoke the immune system without causing illness, is needed for its quality control during the process of development [22]. However, there are certain constraints which need to be monitored to check its efficacy which is a laborious and continuous job. The International Conference on Harmonization (ICH) has developed pre-set criteria (various assays) to regulate the quality of a biopharmaceutical product on current good manufacturing practices (cGMP). To overcome adverse events and to get an overall acceptable level following immunization, risk assessment must be performed during each and every step of vaccine development [23]. The following are the important overviews of the major quality control issues which must be addressed before commercialization of vaccines.

12.3.1 Monitoring Efficacy of Vaccines (Pre-clinical Stage)

Vaccine is a unique complex formulation which is composed of one or more types of immunogenic components including live attenuated or killed viral or bacterial part, proteins, polysaccharide, polynucleotide, and conjugates. Apart from these, there are also some agents added to modify and boost a long-lasting immune response to the body. Vaccine efficacy is defined as the percentage of reduction in the incidence rate of disease and has minimal or no adverse reactions post immunization. So there is always a need not only to monitor the chemical and structural integrity of each constituent but also to ensure that the vaccine is capable of immunogenicity [24]. Safety assessment of vaccine starts from the pre-clinical evaluation of vaccine constituents (for purity, sterility, and stability), followed by the clinical and development phase, and finally the distribution, transportation, and duration of use of vaccines [25]. One of the most important factors is to maintain the shelf life of vaccine during the distribution process. For this bulk, intermediate as well as final container product also needs to be monitored for either maintaining the required

Table 12.2 Licensed vaccines for human use

Disease target	Causative agent	Trade name	Vaccine type	Vaccine components	Adjuvant used
Influenza	Influenza virus	AGRIFLU	Inactivated	Trivalent whole virus based (for types A and B)	None
		FluMist Quadrivalent	Live inactivated	Quadrivalent vaccine (for intranasal spray) contains an A/H1N1 strain, an A/H3N2 strain, and two B strains	
		Influenza A (H1N1) 2009 Monovalent Vaccine	Inactivated against H1N1 pandemic 2009	Monovalent split vaccine whole cell approach	
Tuberculosis	*Mycobacterium tuberculosis*	BCG vaccine "SSI"	Live attenuated	Bacillus Calmette-Guerin (BCG), Danish strain 1331	None
Anthrax	*Bacillus anthracis*	BioThrax	Subunit	Cell-free filtrates of microaerophilic cultures of a virulent, non-encapsulated strain of *Bacillus anthracis* and proteins including protective antigen (PA)	Alum
Cervical cancer	Human papilloma virus (HPV)	Cervarix	Subunit virus-like particle	Recombinant L1 protein, the major antigenic protein of the capsid, of oncogenic HPV types 16 and 18	AS04
		Gardasil		Recombinant quadrivalent vaccine prepared from purified virus-like particles (VLPs) of L1 capsid of HPV type 6, 11, 16, and 18	Alum
Hib-induced disease (pneumonia meningitis)	*Haemophilus influenzae* type B	COMVAX	Conjugate	*Haemophilus influenzae type* b conjugate and hepatitis B vaccine	Alum
Hepatitis B	Hepatitis B virus				
Hepatitis A	Hepatitis A virus	Havix	Inactivated virus	The virus strain (HM175 strain) is propagated in MRC-5 human diploid cells, treated with formalin to inactivate	Alum

(continued)

Table 12.2 (continued)

Disease target	Causative agent	Trade name	Vaccine type	Vaccine components	Adjuvant used
Polio	Poliovirus (types 1, 2, and 3)	IPOL	Live inactivated	Inactivated at +37 °C for at least 12 days with 1:4000 formalin	None
Diphtheria	*Corynebacterium diphtheriae*	Infanrix	Subunit	Toxoid of diphtheria and tetanus and the acellular pertussis antigens (inactivated pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin)	Alum
Tetanus	*Clostridium tetani*				
Pertussis	*Bordetella pertussis*				
Diphtheria	*Corynebacterium diphtheriae*	Pediarix	Subunit	As Infanrix in combination with HBsAg and types 1, 2, and 3 polioviruses	Alum
Tetanus	*Clostridium tetani*				
Pertussis	*Bordetella pertussis*				
Polio	Poliovirus				
Hepatitis	Hepatitis B virus				
Hepatitis B	Hepatitis B virus	Recombivax HB	Subunit viral	Derived from hepatitis B surface antigen (HBsAg)	Alum
Herpes zoster	Herpes zoster virus	Zostavax	Live attenuated	Oka/Merck strain of VZV	

temperature or not. Recalculation of the expiry date is needed after an unintended temperature excursion due to cold storage unit failure or mishandling during transportation [26]. All vaccines are prone to be damaged due to temperature elevation or freezing during distribution, during storage, and even during the use of vaccine [27]. The therapeutic agents (medicines, drugs, vaccines, etc.) and the medical or immunization procedures cannot be declared as totally risk-free but are used to ensure that the benefits are far much more than any potential emerging risk.

The final vaccine product is an ideal defined product (both in composition and structure) produced as a result of high-profile assays performed to monitor the efficacy of vaccine in order to reduce all the issues that are discussed in the above paragraph. The number and type of assays that must be performed depend on the type of vaccine to be prepared. For example, the most crucial assay for live attenuated vaccine development is to calculate the number of pathogenic particles that must be included in a single dose and the screening of impurities in each ingredient. When the product is licensed, the post-licensure test is carried out in the form of survey to know the efficacy and safety level of the vaccines when applied on a community [28]. To monitor the efficacy of vaccines during pre-clinical studies, essays are broadly divided into the following two categories.

12.3.2 Assays for Measuring the Concentration and Integrity of Antigens

To quantify an intermediate and final product as well as impurities in the final product, various colorimetric assays and analytical separation tests are performed. Calorimetric assays are used to measure the concentration of proteins and polysaccharide, number of primary amine groups and host cell DNA, etc. The techniques used to separate the impurity and maintain the integrity and stability of vaccines (protein and polysaccharide in nature) are chromatography (liquid and gas) and gel electrophoresis. Antigen characterization, impurity identification, structural degradation such as oxidation, and fragmentation can be easily measured by mass spectrometry [28]. A list of various physicochemical and immunochemical techniques, along with their details, is given in Table 12.3.

12.3.3 Assays for Measuring Biological Activity, Active Concentration, and Confirmation of Antigen

This part comprises of various assays designed for in vitro and in vivo studies as well as for physicochemical and immunochemical analysis. For the quality assessment of vaccines, both physicochemical and immunochemical evaluations are very crucial to confirm antigenic structure and access immune-dominant epitopes, especially for monoclonal antibody. The integrity of antigenic structure has an important role in vaccine potency and efficacy [29]. In combination vaccines, the quality assessment of individual antigen is a hurdle which must be proven by a correlate protection and

Table 12.3 Physicochemical and immunochemical techniques for vaccine development

Technique	Information regarding	Identity	Purity	Stability
Chromatography				
Reversed phase	Purity, stability, degradation, and protein modification	+	+	+
Ion exchange	Protein modification, degradation, aggregation, and purity	0	+	0
Size exclusion	Hydrodynamic size, aggregation, purity, and oligomeric repartition	0	+	+
Immunochemical techniques				
Biosensor analysis	Antigen concentration, epitope integrity, and binding kinetics	+	0	+
ELISA	Antigen concentration	+	0	+
Immunoblotting	Size, protein modification, degradation, and aggregation	+	0	+
Spectroscopy				
Circular dichroism	Secondary and tertiary structure	−	−	0
Electron microscopy	Imaging of supramolecular structures and integrity	0	0	+
Fluorescence spectroscopy	Tertiary structure of proteins and protein unfolding and refolding	−	−	0
Infrared spectroscopy	Excipients and protein structure	−	0	−
Light scattering	Aggregation	−	−	+
Nuclear magnetic resonance	Excipients and polysaccharide structures	−	0	−
UV absorbance spectroscopy	Tertiary structure of proteins, protein unfolding and refolding, and aggregation	−	−	+
Electrophoresis				
Capillary electrophoresis	Primary structure of proteins and protein degradation	0	0	+
Isoelectric focusing (IEF)	Isoelectric point (pI), protein modifications, degradation, aggregation, and purity	0	+	+
PAGE (SDS or native)	Size of protein, protein modifications, degradation, aggregation, and purity	0	+	+
2D electrophoresis (IEF plus SDS-PAGE)	Size, pl, protein modification, degradation, aggregation, and profile of protein impurities	+	+	+
Mass spectrometry				
	Primary structure of proteins, protein modification, and degradation	+	0	+
Differential scanning calorimetry				
	Thermodynamics of protein unfolding	−	−	+

+, yes; 0, neutral/possibly; −: no/unfavorable

efficacy both in in vitro and in vivo studies as well as immune chemical and serological studies [30, 31]. In vivo studies are a platform which provides an ideal outcome from immune response parameters including innate, cellular, and humoral immunity with protection. There are some restrictions especially to develop animal models for those human diseases which have specific range of surface proteins that interact with specific host receptors [32]. Keeping in view, it is acknowledged that the ability and safety of pre-clinical studies for the product which is human specific will be limited in the clinical trials. For this, transgenic mice with specific human receptors such as CD46 and CD66 have been developed which have now been used to develop novel human vaccines [33]. Similarly, humanized immune-deficient mice are also developed in which human tissues with immunological functions are transplanted into mice and studied [34].

12.3.4 Monitoring Efficacy of Vaccines (Clinical Stage)

To move from a pre-clinical stage (in vitro and in vivo) into a clinical stage (first human test) in vaccine development is anyhow a difficult decision. For this, the Committee for Medicinal Products for Human Use (CHMP) has developed certain guidelines which should be adopted before moving from pre-clinical to clinical trials [35]. The most important of these guidelines are some of the serious adverse reactions (discussed below) and their remedies, which must be kept in mind before moving to a clinical trial. To evaluate the efficacy of vaccines, serum antibody titer is checked in the clinical trials and is acceptable if it is above a certain threshold level. Similarly, cellular immunity measurement is also recommended to evaluate both quantitative and qualitative analyses of T cells, such as Th1, Th2, T memory cells, and relevant cytokines.

12.3.5 Adverse Events Following Immunization (AEFI)

Unlike medicines, vaccines are administered in bulk and mostly to the healthy population, such as children and infants, which possess low tolerance against potential risk or side effects of vaccines. As we have discussed, vaccines are composed of somehow a pathogenic agent which may evert and, apart from giving long-term immunization, may cause some discomfort and even death. As the name indicates, AEFI are the unwanted adverse events which occur after immediate or weeks after immunization irrespective of whether it is due to vaccines or not. Some of the AEFI are summarized in Table 12.4.

The Centers for Disease Control and Prevention (CDC) and the US Food and Drug Administration (FDA) are assigned to conduct and evaluate the safety as well as the efficacy of each vaccine post licensed, during various steps of distribution and after immunization [36, 37]. The CDC and FDA after evaluation submit the Vaccine Adverse Event Reporting System (VAERS) which is a passive (spontaneous) reporting system as shown in Fig. 12.2. The report is passively made based on the

Table 12.4 Various adverse events following immunization (AEFI)

AEFI	Details
Vaccines induced or vaccine reaction events	Some events caused post correct vaccination such as pain, swelling, redness, and even fever
	Inherent properties of vaccine such as presence of an adjuvant sometime causing local inflammatory response or eversion of live attenuated vaccines including mild fever and/or rash for about 10 days post immunization (MMR and poliovirus vaccines)
Immunization errors	Errors or mistakes made during preparation, processing, transport, handling, and administration of vaccines, e.g., injecting a fully liquid without reconstitution or administration of oral vaccine intramuscularly
Coincidental events	Immediate events followed by vaccination but not caused by vaccines such as flu-like symptoms, etc.
Immunization anxiety reaction	Anxiety reaction about or pain from the injection itself like syncope and panic attack after vaccination
Vaccine failure	Many reasons as mentioned right from the preparation till to the end users like failure in the cold chain requirements
Unknown	Difficulty to know about the cause

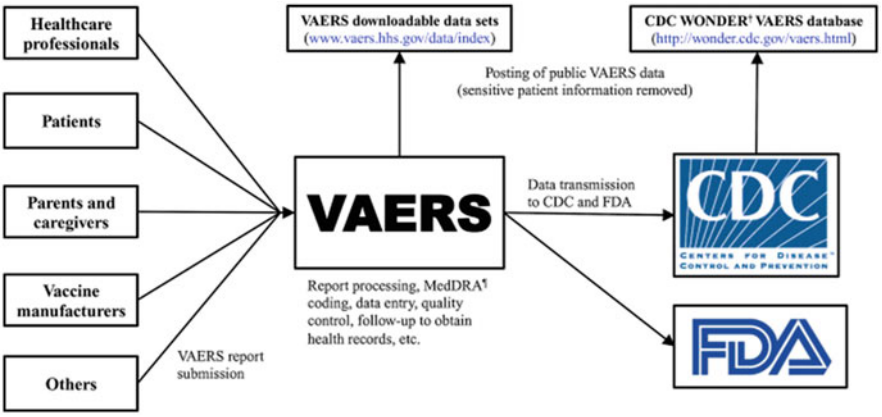

Fig. 12.2 Data flow diagram of Vaccine Adverse Event and Reporting System (VAERS) submission. During 2011–2014, healthcare professionals, patients and parents, vaccine manufacturers, and others (friends of parents, third-party reporter, etc.) submitted 38%, 14%, 30%, and 12% of reports (Adopted from reference [36])

volunteers who voluntarily share their experience, not based to identify and collect information. They also rely on the healthcare professionals, patients, parents, and caregivers and also on the health record surveillance registered system [38, 39].

12.3.6 Risk Factors Related with Vaccination

As highlighted in the above discussion, importance and benefits of vaccines could not be ignored, but there are various risk factors which need to be considered before immunization.

- Febrile convulsion, sometimes when a new vaccine is going to be introduced due to an identified risk for vaccines such as quadrivalent vaccines (e.g., measles-mumps-rubella and varicella vaccines).
- Spontaneous abortion risk, without identifying and investigating a potential risk prior to registration, such as when adjuvanted papilloma virus vaccines are administered without the knowledge of pregnancy.
- Immune-mediated disease risk, e.g., adjuvanted vaccination, such as adjuvant human papillomavirus vaccine and adjuvanted influenza vaccines.
- Immune-compromised pregnant individual, e.g., administration of adjuvanted vaccines to new target population where safety data is scarce.
- There is always a risk when introducing new vaccines, and the available infrastructure for pharmacovigilance is limited, e.g., for malaria vaccines and dengue vaccines in Africa and developing countries, respectively.

12.4 Vaccine Kinetics and Stability

Vaccine kinetics are typically used to determine the estimated degradation rates of vaccines using accelerated stability programs exposing the product to grater temperature than those recommended for storage of vaccines [40]. Immune system works against the pathogen and recognize pathogen characteristics. Therefore, characteristics of the pathogens are studied to increase the efficacy of vaccines, such as dimension of pathogens and specific PAMPs used as adjuvants for immune stimulation [41]. Vaccine stability is the most important step in vaccine efficacy and is a major concern associated with vaccine distribution. To study the stability of vaccine, the following two essential approaches are used.

12.4.1 Temperature Ramping Experiment

It involves the monitoring of changes that occur in biophysical properties of vaccine when the temperature is increased at a specific rate. These include monitoring the decomposition extent and/or some parameters related with thermodynamics including enthalpy and free energy. These experiments are performed very quickly (few hours) and are therefore used in vaccine formulation, development, and screening of stabilizing conditions [42, 43]. Besides thermodynamics, kinetics is also necessary to evaluate the stability of the vaccines. Kinetic stability is inversely proportional to the degradation rate, i.e., when the kinetic stability is high, the degradation rate is low and vice versa.

12.4.2 Accelerated Stability Studies

This study is performed to find out the degradation rate of vaccines over time because of exposure to high environmental temperature than the required recommended temperature for vaccine processing and storage. Liquid chromatography, gel electrophoresis, various immunochemical assays, and, to find out the antigenic titer, various methods are used to investigate the vaccine degradation rate at different time intervals [26, 44]. These studies are also used as precautionary measures to determine stabilized conditions, to enhance the potency of manufacturing process, and to estimate the shelf life of vaccines.

From the last couple of decades, medical field has made significant advances especially in the drug delivery system. The development of controlled release system is one of its great achievements. The main objectives of this system are to check the stability and provide the concentration of the vaccine in circulation or to the specific target site at a desired amount as far as possible [45], in a control release rate and time [46]. For vaccine stability, the controlled system initially releases the vaccine contents to attain the effective therapeutic concentration quickly followed by the release kinetics to provide the maintenance concentration. The main objective behind this is to increase the efficient availability, effectiveness, and stability and to release more effective product. So to find out and predict the kinetics of a vaccine, various mathematical models are used to compare all the phenomena that affect vaccine release kinetics [47].

12.4.3 Kinetic Model (Zero or First Order)

Kinetic model is the common accelerated stability model method used to analyze the data obtained and estimate long-term stability of a vaccine. To determine the constant rate for two or more temperatures, the data obtained from the accelerated stability studies are fit with a simple kinetic model, which is typically zero- or first-order kinetic models. Following experiment, an unknown constant rate is calculated from the data at a specific temperature using Arrhenius dependence of the reaction rate [44, 48]. Based on applications of zero- or first-order kinetics, stability estimation seems too simple for description of biological products, which commonly have complex and multistep degradation reactions [49].

12.4.4 Sophisticated Degradation Kinetic Models (Two-Step Model)

In this type of kinetic models, a two-step kinetic model is used such as an *n*th order and an autocatalytic component to investigate the degradation rate of biological products. Examples of this complicated process include Fulcher-Tammann equation or Prout-Tompkins nucleation models [50, 51]. This is a superior kinetic model which suitably mimics and investigates the complicated process of biological product decomposition [52].

Relatively the *n*th-order kinetic model is best fitted to calculate the degradation rate for an adjuvanted protein vaccine, two-step model for live virus vaccine, and an autocatalytic-type kinetic model for an oil-in-water adjuvant formulation [48]. Based on the experimental data and kinetic analysis, it has been estimated that there is approximately five percentage point difference from that of actual value for long-term storage conditions, post excursions of temperature, and during shipments of freeze-dried products [40].

12.5 Conclusion

So far prophylactic vaccination has shown a profound impact on public health sector by decreasing disease incidence rate; still there is a need of novel vaccine development to combat and defeat the novel infections. Safety and efficacy of vaccines are the major concerns which must be evaluated during pre-clinical (in vitro and in vivo), clinical (first human trial), and developmental trial phases to avoid adverse reactions following vaccination. This is quite a lengthy exercise which needs contribution from various research and developmental expertise, quality control and assurance, safe production, marketing, and sale. This chapter highlights various aspects and types of immunity and vaccines, different assays used to evaluate the safety and efficacy concerns, and various kinetic models to know the degradation rates of vaccines at different time points.

References

1. Riedel S (ed) (2005) Edward Jenner and the history of smallpox and vaccination. In: Baylor University Medical Center Proceedings. Taylor & Francis
2. Doherty M, Buchy P, Standaert B, Giaquinto C, Prado-Cohrs D (2016) Vaccine impact: benefits for human health. Vaccine 34(52):6707–6714
3. Plotkin SL, Plotkin SA (2004) A short history of vaccination. Vaccine 5:1–16
4. Health UDo, Services H (2008) National institute of allergy and infectious diseases. In: Understanding vaccines: what they are, how they work. National Institute of Allergy and Infectious Diseases Website
5. Hanquet G, Valenciano M, Simondon F, Moren A (2013) Vaccine effects and impact of vaccination programmes in post-licensure studies. Vaccine 31(48):5634–5642
6. Haber M, LonginiI JRIM, Halloran ME (1991) Measures of the effects of vaccination in a randomly mixing population. Int J Epidemiol 20(1):300–310
7. Sell S, Max EE, Berkower I (2001) Immunology, immunopathology and immunity. ASM Press, Washington, DC
8. Clem AS (2011) Fundamentals of vaccine immunology. J Global Infect Dis 3(1):73
9. Hartshorn KL, White MR, Crouch EC (2002) Contributions of the N-and C-terminal domains of surfactant protein d to the binding, aggregation, and phagocytic uptake of bacteria. Infect Immun 70(11):6129–6139
10. Margolick JB, Markham RB, Scott AL (2014) The immune system and host defense against infections. Infect Dis Epidemiol Theory Pract:317–343
11. Sarma JV, Ward PA (2011) The complement system. Cell Tissue Res 343(1):227–235

12. Janssens S, Beyaert R (2003) Role of Toll-like receptors in pathogen recognition. Clin Microbiol Rev 16(4):637–646
13. Pashine A, Valiante NM, Ulmer JB (2005) Targeting the innate immune response with improved vaccine adjuvants. Nat Med 11(4s):S63
14. Nossal GJ (1987) The basic components of the immune system. N Engl J Med 316 (21):1320–1325
15. Sun H, Sun R, Tian Z (2017) Components of the immune system. In: Practical immunodermatology. Springer, pp 3–22
16. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA (2017) Fundamentals and methods for T-and B-cell epitope prediction. J Immunol Res 2017
17. Annunziato F, Romagnani C, Romagnani S (2015) The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 135(3):626–635
18. Jones LD, Thompson AB, Moody MA (2019) Vaccine development. In: Genomic and precision medicine. Elsevier, pp 167–183
19. Duggan AT, Perdomo MF, Piombino-Mascali D, Marciniak S, Poinar D, Emery MV et al (2016) 17th century variola virus reveals the recent history of smallpox. Curr Biol 26 (24):3407–3412
20. Barquet N, Domingo P (1997) Smallpox: the triumph over the most terrible of the ministers of death. Ann Intern Med 127(8_Part_1):635–642
21. Underwood EA (1949) Edward Jenner, Benjamin Waterhouse, and the introduction of vaccination into the United States. Nature 163(4152):823
22. Sheridan C (2005) The business of making vaccines. Nat Biotechnol 23(11):1359–1366
23. Teasdale A, Elder D, Nims RW (2017) ICH quality guidelines. Wiley Online Library
24. Volkin D, Burke C, Sanyal G, Middaugh C (1996) Analysis of vaccine stability. Dev Biol Stand 87:135–142
25. Di Pasquale A, Bonanni P, Garçon N, Stanberry LR, El-Hodhod M, Da Silva FT (2016) Vaccine safety evaluation: practical aspects in assessing benefits and risks. Vaccine 34 (52):6672–6680
26. Schofield TL (2009) Vaccine stability study design and analysis to support product licensure. Biologicals 37(6):387–396
27. Kristensen D, Chen D, Cummings R (2011) Vaccine stabilization: research, commercialization, and potential impact. Vaccine 29(41):7122–7124
28. Metz B, Van Den Dobbelsteen G, Van Els C, Van Der Gun J, Levels L, Van Der Pol L et al (2009) Quality-control issues and approaches in vaccine development. Expert Rev Vaccines 8 (2):227–238
29. Edwards KM (2001) Development, acceptance, and use of immunologic correlates of protection in monitoring the effectiveness of combination vaccines. Clin Infect Dis 33(Supplement_4): S274–S2S7
30. Taffs RE (2001) Potency tests of combination vaccines. Clin Infect Dis 33(Supplement_4): S362–S3S6
31. Postema AS, Myers MG, Breiman RF (2001) Challenges in the development, licensure, and use of combination vaccines. Clin Infect Dis 33(Supplement_4):S261–S2S6
32. Gorringe A, Reddin K, Funnell S, Johansson L, Rytkönen A, Jonsson A-B (2005) Experimental disease models for the assessment of meningococcal vaccines. Vaccine 23(17–18):2214–2217
33. Johansson L, Rytkönen A, Bergman P, Albiger B, Källström H, Hökfelt T et al (2003) CD46 in meningococcal disease. Science 301(5631):373–375
34. Nomura T, Tamaoki N, Takakura A, Suemizu H (2008) Basic concept of development and practical application of animal models for human diseases. In: Humanized mice. Springer, pp 1–24
35. Ale T. Operational guidelines. 2015.
36. Shimabukuro TT, Nguyen M, Martin D, DeStefano F (2015) Safety monitoring in the vaccine adverse event reporting system (VAERS). Vaccine 33(36):4398–4405

37. Varricchio F, Iskander J, Destefano F, Ball R, Pless R, Braun MM et al (2004) Understanding vaccine safety information from the vaccine adverse event reporting system. Pediatr Infect Dis J 23(4):287–294
38. Baggs J, Gee J, Lewis E, Fowler G, Benson P, Lieu T et al (2011) The Vaccine Safety Datalink: a model for monitoring immunization safety. Pediatrics 127(Supplement 1):S45–S53
39. Baker MA, Nguyen M, Cole DV, Lee GM, Lieu TA (2013) Post-licensure rapid immunization safety monitoring program (PRISM) data characterization. Vaccine 31:K98–K112
40. Clénet D (2018) Accurate prediction of vaccine stability under real storage conditions and during temperature excursions. Eur J Pharm Biopharm 125:76–84
41. Johansen P, Storni T, Rettig L, Qiu Z, Der-Sarkissian A, Smith KA et al (2008) Antigen kinetics determines immune reactivity. Proc Natl Acad Sci 105(13):5189–5194
42. Hu L, Joshi SB, Liyanage MR, Pansalawatta M, Alderson MR, Tate A et al (2013) Physical characterization and formulation development of a recombinant pneumolysoid protein-based pneumococcal vaccine. J Pharm Sci 102(2):387–400
43. Chiu MH, Prenner EJ (2011) Differential scanning calorimetry: an invaluable tool for a detailed thermodynamic characterization of macromolecules and their interactions. J Pharm Bioallied Sci 3(1):39
44. Evans RK, Nawrocki DK, Isopi LA, Williams DM, Casimiro DR, Chin S et al (2004) Development of stable liquid formulations for adenovirus-based vaccines. J Pharm Sci 93 (10):2458–2475
45. Langer RS, Wise DL (2019) Medical applications of controlled release. CRC Press
46. Senapati S, Mahanta AK, Kumar S, Maiti P (2018) Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther 3(1):1–19
47. Kydonieus AF (2017) Treatise on controlled drug delivery: fundamentals-optimization-applications. Routledge
48. Clénet D, Imbert F, Probeck P, Rahman N, Ausar SF (2014) Advanced kinetic analysis as a tool for formulation development and prediction of vaccine stability. J Pharm Sci 103 (10):3055–3064
49. Noël C, Charles S, Françon A, Flandrois JP (2001) A mathematical model describing the thermal virus inactivation. Vaccine 19(25–26):3575–3582
50. Oliva A, Fariña J, Llabrés M (1996) Influence of temperature and shaking on stability of insulin preparations: degradation kinetics. Int J Pharm 143(2):163–170
51. Šimon P (2011) Fourty years of the Šesták–Berggren equation. Thermochim Acta 520 (1–2):156–157
52. Roduit B, Hartmann M, Folly P, Sarbach A, Baltensperger R (2014) Prediction of thermal stability of materials by modified kinetic and model selection approaches based on limited amount of experimental points. Thermochim Acta 579:31–39