Asian Journal of Biomedical and Pharmaceutical Sciences *ISSN 2249 – 622X*

Emerging functional assays used for vaccine efficiency.

Pratik Zagade*, Nigade G

Department of Pharmaceutical Chemistry, SGR Sable College of Pharmacy, Pune, India

Abstract

In the countries of the west, meningococcal disease is frequently caused by Neisseria meningitidis. Major to increase a secure and efficacious vaccine towards this serogroup which is appropriate for use in infants and younger children. After vaccination with candidate vaccines, to determine the high-quality of the immune response laboratory correlates of safety are needed. For serogroups A and C, Serum Bactericidal Assay (SBA) is a well-set up predictor for safety, however for serogroup B different mechanisms besides SBA can also be worried in conferring safety from disease. The general SBAs are labor intensive and the variations in protocols among specific laboratories make interpretation of outcomes difficult. Hence, the SBA assays with a colorimetric and a fluorometric end point within the presence of aamarblue were selected and compared to a well-known conventional SBA assay, wherein colony counts are performed to evaluate the titer. A colorimetric end point required a spectrophotometer, while a fluorometric end point required a fluorometer. Several laboratory strategies for recognition and evaluation of the immunogenicity of possible vaccine antigens are described in this review.

Keywords: Meningococcal disease, Immune response, Colorimetric end point, Titer, Vaccine antigen.

Accepted on 07 July, 2021

Introduction

The word "Vaccine" is arises from the latin word Variolae vaccinae In 1798 the english physician, edward jenner demonstrated the prevention of small pox in humans. These days the term 'vaccine' applies to any or all biological preparations made from living organisms, which enhance immunity against disease and either stop (prophylactic vaccines) or, in some cases, treat disease (therapeutic vaccines). The route of administration of vaccine is in liquid form, either by injection, by oral, or by intranasal routes.

Literature Review

The vaccines are composed of either the disease-causing microorganism or the components of the same. It may be constructed in several ways.

• The vaccine can be prepared by using living organisms by altering its antigenicity, usually by genetic modification to reduce their ability to cause disease or from cultivation under sub-optimal conditions (also called attenuation) [1].

• From whole organisms that have been inactivated by thermal, chemical or other means.

• From components of the disease-causing organism, like specific proteins and polysaccharides, or nucleic acids.

• From inactivated toxins of toxin-producing bacteria.

• From the conjugation (linkage) of polysaccharides to proteins (this increases the effectiveness of polysaccharide vaccines in young children.

Effect of vaccine on immune system

An immunological reaction is triggered when inactivated or damaged disease-causing microorganisms enter the body. This response is a reflection of the body's natural response to infection (Figure 1).

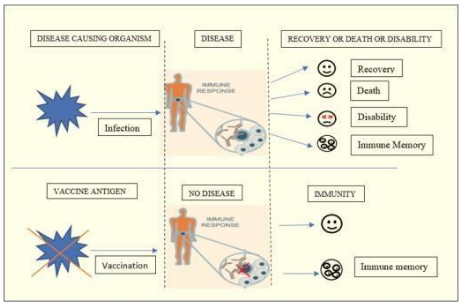

Figure 1. *Comparison of the immune response to a diseases-causing organism and to a vaccine.*

The components of disease-causing species or vaccine components that cause an immunological reaction are referred to as "antigens." These antigens activate the immune system's production of antibodies. Antibodies bind to associated antigens and activate their degradation by alternate immune cells.

The induced immunologic response to either a disease-causing organism or to a vaccine configures immune cells of our body is efficient for quickly recognizing, reacting to, and moderate the appropriate disease-causing organism [2]. If the person is later exposed to the same disease-causing organism, the immune system can contain antibodies and eliminate the infection before it will cause damage to the body.

Efficacy of vaccine

Efficacy of Vaccine is referred to the reduction in incidence of disease amongst individuals those are vaccinated related to the incidence within the unvaccinated. Vaccines might fail to induce immunity in a few individuals. However, the foremost effective vaccines induce a protecting immunologic response in >95% of individuals. The reason behind this is inherently variability, individuals don't respond identically to vaccines.

The disease transmission is depends upon high level of vaccination coverage, as the efficient vaccine is achieved the transmission of disease is interrupted. The impact of herd immunity thought as when disease transmission is interrupted within those individuals who were not vaccinated, or WHO were vaccinated and failed to develop immunity, are protected against disease. Disease transmission in a partially immune population given in (Figure 2).

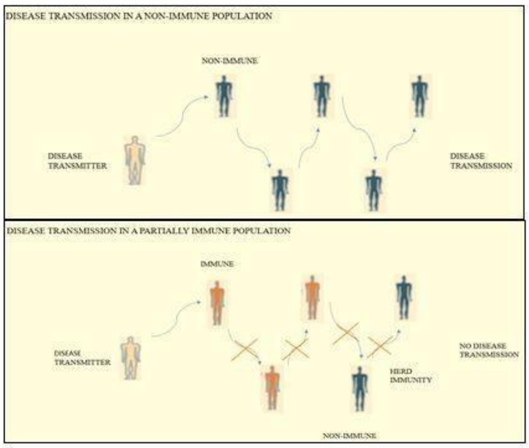

Figure 2. *Herd immunity.*

The standard of vaccine coverage required to break the spread of the disease would depend on following facts:

• Lack of difficulty in transmission of disease

• The effectiveness of that particular vaccine at their stimulating immunity.

The proportion of healthy individuals in a population that can inhibit the transmission of the disease is known as the herd immunity threshold. Every disease has its own level for herd immunity. The more quickly reproduced or transferred the infection, the greater the threshold. The higher the threshold, the greater the vaccine efficacy needed to stop disease transmission along with their coverage percentage. Extremely quickly transmitted infections, such as measles, will continue to occur in the population even though the effectiveness and availability of vaccines are very high.

Efficiency of vaccine

Vaccine Performance calculates the decrease in the risk of disease in the vaccinated population relative to the risk of disease in the non-vaccinated population. In terms of epidemiology, it is described as the variation between the unvaccinated attack rate of the disease and the unvaccinated attack rate of the vaccine [3,4].

When categorized into Unvaccinated and Vaccinated groups, vaccine efficiency is calculated as

Vaccine Efficiency=(Attack rate in the Unvaccinated-Attack rate in the Vaccinated)/Attack rate in the Unvaccinated ×100.

Vaccine effectiveness is depending upon vaccine efficacy. The performance of vaccine under field conditions (usually retrospectively) is measured from vaccine effectiveness, whereas the performance of vaccine under study conditions (usually prospectively) is measured from vaccine efficiency. Therefore, the vaccine effectiveness will not on the performance of the vaccine delivery program but also the performance of the vaccine. Furthermore, whereas prevention of disease is depending upon vaccine efficiency and vaccine effectiveness can estimate the ability of a vaccine to prevent a specific conclusion. For example, medication or death from a specific disease.

Complement system

The experiment that belongs to Complement while working within the laboratory. It is the heat-labile protein of ordinary plasma, which reasons the opsonization. A manner wherein pathogens are included with a substance called an opsonin, marking the pathogen out for destruction by the immune system and killing of bacteria.

The complement system includes a chain of heat-labile serum proteins. These enhances exist as soluble inactive precursors that when activated; a complement issue may then act as an enzyme. Enzymatic chain reactions of complement protein referred to as cascade reactions and frequently need a "trigger" to initiate the reaction chain. This is a sequence of enzymes whose activation ultimately results in the breakdown of cellular membrane and also in the death of cells or invading microorganisms. Complement is a vital part of the body defense system [5].

Complement needs to be present for measure the ability of vaccine-induced antibodies to promote bacterial killing *in vitro*. The serum were collected from vaccinated individuals is processed to preserve complement and in some assays performed using intrinsic or endogenous complement or an external source of complement. For measurement of actual titter different assays can be performed, the concentration of the complement with an internal complement is decreased by a dilution of 1:8 (12.5 per cent complement in the SBA reaction) if the addition of an additional complement is implemented. When another type of complement is being used, it must be collected from healthy persons whose serum loses bactericidal action against the strain to be tested. In order to achieve a complement lacking of naturally produced antibodies, it is important to monitor sera from a variety of individuals to identify those that lack bacterial action against the strain studied.

Meningococcal fHbp is species-specific and is limited to human fH. Rabbit fH does not bind to the protein of meningococcal fH. Therefore, when the rabbit substitute is included in the respective assay process, no human fH is bound to the bacteria, the alternate pathway is no longer in check, As a result, complement-mediated bacterial lyse is not blocked and the bacteria are more vulnerable to complement-mediated destruction. This means that the titres are significantly greater in the rSBA as less antibodies are required to kill the much more bacterial invasion.

Serum Bactericidal Assay (SBA)

To studies the bacterial activity of the patient's bloodstream during antimicrobial treatment, the bacterial pathogen that was isolated from the patient, serum bactericidal test is used. It is modification of broth dilution method. This is classified as *in vitro* tests regulates, in the clinical microbiology laboratory, which involves the association between the pathogen, the antimicrobial agent and the patient [6].

In 1960's, to measure meningococcal killing Serum bactericidal assay was originated. During an outbreak of invasive meningococcal serogroup C disease at an army base, Gold Schneider and coworkers measured functional antibody concentrations in newly-enlisted military recruits. In addition, recruits with a given amount of circulating functional meningococcal antibodies were found to be at the lowest risk of disease, whereas recruits with antibody levels below this concentration were at the highest risk. The group used the SBA (Sometimes used as the Bactericidal Activity test or BCA in the literature) assay to measure the concentration of these functional antibodies was the SBA that they have a tendency to take "SBA" to mean "Serum Bactericidal Antibody". This assay assesses the ability of the antibodies to denature bacteria in complement presence. Serum from both normal recruits and the others in their examination of the recruits World Health Organization succumbed to disease was used. The minimum titer that correlates with protection was the dilution 1:4.

The SBA assay tests the ability of circulating antibodies to lyse meningococci *via* means of complement, generally known as complement-mediated killing *via* the classical immune response pathway. The bactericidal titer is characterized as the dilution of the test serum resulting in a decrease of at least 50 per cent in Colony Forming Units (CFUs) per mL of bacteria. As such, this is one of the functional assays in that it measures the killing of live bacteria. The circumstances the test has a major effect on the outcomes, so it is important to use specified and validated conditions, defined by a team of experts appointed by the WHO. In the course of development of meningococcal vaccines, the recommendations for screening required the use of baby rabbit complement due to the shortage of this reagent and the difficulty of obtaining human complement [7].

For evaluation of meningococcal vaccine efficacy, the Serum Bactericidal Antibody (SBA) assay has become a surrogate method. It is employed as a measure of vaccine-induced antibody performs in conjunction with complement to kill bacteria. The assay will provide information on whether or not a particular serum sample has a level (titer) of bactericidal antibodies adequate to achieve a protecting threshold. The percentage Seropositivity (%SP) of subjects surpassing the protective threshold can also provide by the SBA assays. Serum reactivity against varied bacterial isolates is evaluated using the standard viable count technique. SBA has been used to estimate immunogenicity induced by vaccines for protection against some infectious diseases like cholera, typhoid, and meningococcal disease.

The Serum Bactericidal Assay (SBA) was used to assess bactericidal antibody rates, and also an increase in titers correlates with their protection after immunization with the serogroup A and C capsular polysaccharide vaccines. The SBA has also been used to determine the effectiveness of serogroup B vaccines consisting primarily of outer membrane vesicles instead of capsular polysaccharides, but the association between efficacy and safety has been weak in some cases. The SBA measures only the humoral response and therefore does not calculate the phagocytosis contribution which may be significant for determining immune response to serogroup B vaccines.

The SBA has assumed multiple perspectives; it means constantly bacterial cell incubation, immune serum, and a source of supplement for specific time periods, and in some fashion, quantifying bacterial cell death. The way this can be done has varied according to photometric. Classic colony counting methods include traditional or microliter plates. Although the classic method of plating and colony counting is commonly used, it is very complex, labor intensive, expensive and susceptible to errors during colonial dilution and counting.

Studies testing the effectiveness of meningococcal vaccines would require impractically significant sample sizes, as the meningococcal disease is actually extremely rare. For this purpose it was important to establish a surrogate measure which would promote the determination of efficacy. In 1969 his associates published a seminal paper explaining the use of SBA tests and the correlation of the results with meningococcal susceptibility. In order to execute the SBA assay, sera samples was serially diluted from the respective subjects or individuals and incubated with a suspension containing a specified meningococcal strain; in most experiments, an exogenous human complement lacking bactericidal activity was then added to the test strains.

Bactericidal action of any disease was dependent on many factors; these being the efficiency of bacterial killing relative to controls, and others identified dilution of sera with SBA titers at which 50 percent of killing occurred. However, the investigators tested sera from newly recruited military recruits for SBA infection against emerging serogroup C meningococcal strains that eventually affected 54 recruits; (5.6 percent of cases and 82.2 percent of randomly chosen men in the same selection panel for each case) had sera with SBA titers with ≥ 4. This analysis revealed that SBA titers ≥ 4 may be predictive of serogroup C IMD resistance. Much higher numbers of controls compared to serogroup C cases have had SBA titers ≥ 4 against serogroup A and B strains, suggesting that, in addition to capsular polysaccharides, SBA action was regulated by subcapsular antigens [8].

The prevalence of SBA titers against particular strains from serogroups A, B and C as being inversely proportional to the occurrence of IMD (Invasive Meningococcal Disease) across age ranges (over 26 years of age), providing further support for the association of SBA titers between ≥ 4 and IMD protection. Since assessing the efficacy of meningococcal vaccines in clinical trials is inefficient due to the low occurrence rate of IMD, the SBA test has become especially useful as a surrogate indicator of the vaccine effectiveness. Many years after associates released the studies, the World Health Organization (WHO) stipulated the use of SBA assays using baby rabbit complement to show effectiveness for future approval of meningococcal polysaccharide vaccines aimed at serogroups A and C.

To measure the bactericidal activity of immune serum the own assays evaluated by many researchers. In 1976, the WHO expert committee on biological standardization proposed a microliter-based assay for the measurement of serum bactericidal activity. Also, 1996 evaluated and optimized assay parameters for intralaboratory reproducibility. They also established the interlaboratory reproducibility of the standardized assay through a multilaboratory study.

Briefly, coworkers had standardized the procedure for serum bactericidal assay is as follows-25 µl of solution in a 96-well microtiter plate, from the 2nd to 11th well columns. Add additionally 25 µl of serum samples is placed in each route's first well, allowing a twofold dilution. Next, bacterial suspension 12.5 µl and 12.5 µl human serum has been added as a complement source. The plate was incubated at 37°C for 30 min without any CO_2. After incubation, the whole plate was they filled with Mueller-Hinton agar (150 µl/well). At the end, the plate was incubated overnight at 37°C with 5% CO_2, and the stereoscopic microscope was used to count the colonies in all wells.

The outcomes were represented as less than or equal to 50% survival of the reciprocal serum dilution relative to the bacterial counts from control wells containing only complement and bacteria. Furthermore, when examined for anti-N meningitidis group B antibodies, they used human serum as a source of complement from a cuban blood bank and confirmed negative [9].

Evaluate serum bactericidal antibody assays for Haemophilus influenza (Hib). As described by for Hib, which is a modification of the process followed serotype a SBA assays (with a viability endpoint and a fluorometric endpoint) were performed. SBA assays had been carried out on 4 separate days for every strain through two independent researchers blinded to the assay results the usage of another method. Ten negative-control seram with poor SBA titers using the reference strain AK1435C3 had been used but were not included within the comparison of traces or the final analysis.

Serial dilutions of sera are incubated with incubated with target bacterial strains to execute this standard SBA assay. As conventional SBA assay is labor-intensive, time-consuming, and unsuitable for analyzing large serum sample numbers. Although automated colony counting may be used, the process is mostly implemented manually and inter-operator inconsistency is a known cause of error when a clinical trial is accredited. SBA methods within laboratories proved difficult to standardize, although with the cumbersomeness of the process, a reliable and high-throughput assay is desperately required for a quick and effective evaluation of the presumed potency of the vaccine. For this purpose, fluorescence-based and colorimetric-SBA assays to substitute CFU plate counting have been actually introduced. Recognizing serum bactericidal assay complement using human and baby rabbit (Figure 3) [10-12].

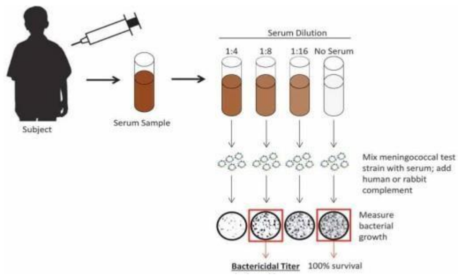

Figure 3. *Schematic recognizing serum bactericidal assay complement using human and baby rabbit.*

Colorimetric Serum Bactericidal Assay (CSBA)

Colorimetric serum bactericidal test i.e. CSBA dependent on the potency for the intake of glucose contributing to acid production is an alternative to prevent the laborious and subjective task of plating and colony counting. In this assay glucose and a pH indicator were added at last in order to estimate the growth of SBA target living cells through color change. Such new guidelines require investigators to approximate serum bacterial activity against without the restriction of the conventional SBA. Precision was determined for CSBA during the validation process.

The colorimetric SBA depends upon the ability of viable bacteria to consume glucose, resulting in acid production, thus reducing the pH that can be identified by a change in the color. Fluorescence SBA uses a reduction-oxidation measure to identify living bacteria, and both measures are often read visually or by a spectrophotometric plate reader. The titers obtained by both assays were closely correlated with the titers obtained by the traditional SBA test. This makes the assays a fast, simple and more accurate alternative to the conventional SBA, making it more suited for large-scale research.

Standardized neisseria meningitides serogroup B Colorimetric Serum Bactericidal Assay. The major limitation of typical SBAs, the counts of the colony cannot be standardized, so it's hard to standardize, labor intensive, and inconvenient. In this study, they optimized the parameters for the intra-laboratory reproducibility of a serogroup BN. Meningitides CSBA and the compared it to a traditional mSBA. They had followed the same procedure of SBA but after incubation, 150 µl of Mueller-Hinton Broth (Oxoid) (MHB) containing 2% of glucose (BDH Laboratory Supplies, England),Vancomycin-

Colimycin-Nystatin (VCN) inhibitor (bioMerieux SA) and 2% bromocresol purple each well has been added. The VCN inhibitor was being used to help make sure the absence of contaminant microorganisms which would influence the results of the assay [12].

Certain operations of the CSBA have been done in biosafety cabinets. The research strain was streaked in isolated colonies and incubated overnight at 37°C with 5 per cent CO_2 in Mueller-Hinton (Oxoid)-10 per cent fetal bovine serum (MH-FBS) agar plates. The strain was sub-cultivated by the spread-plate system and incubated at 37°C for 4 h with 5% CO_2 . They cell suspension was prepared in physiological saline pH 7.2 to 7.4 and Optical Density (OD) was measured at 600 nm. The optimal cell density should be between 0.5 to 0.55. The cell suspension was diluted to yield 100 CFU/12.5µl. First of all, heat-inactivated serum samples (25 ul) were serially diluted two fold in Hanks' Balanced Salt Solution (HBSS; Flow Laboratories, Irvine, Scotland) containing 0.1% Bovine Serum Albumin (BSA;Sigma),the pH of this solution were 7.2 (HBSS-BSA), in 96-well containing flat-bottom tissue culture-treated plates. The 12.5 µl cell suspension and 12.5 µl complement were added. The final concentration of complement in each well was should be 25%. Control wells on each microtiter plate will contained (i) bacteria and buffer (suspension control), (ii) buffer, bacteria, and complement (complement-dependent control), and (iii) heat-inactivated test serum, bacteria, and buffer (complement-independent control). The positive control was kept by using known titer serum was included on each plate. The final volume in each well was 50 µl. The first reciprocal serum dilution also because the final dilution of the complement-independent serum control [11].

The microtiter plate was incubated at 37°C without CO_2 for 30 min. After incubation process, 150 µl of Mueller-Hinton Broth (Oxoid) (MHB) containing 2% glucose (BDH Laboratory Supplies, Poole, England), 2% bromocresol purple, and Vancomycin-Colimycin-Nystatin (VCN) inhibitor (bioMerieux SA, Marcy I'Etoile, France) were added to each well. The VCN inhibitor was used to ensure the absence of contaminant that could affect the results of the assay. The plate was further incubated for 20 h at 37°C with 5% CO_2 . The color change was detected visually as well as spectrophotometrically in a microplate reader at 405 nm. This equipment was placed into the safety cabinet because in the final step there are still viable broth cultures.

The bactericidal titer within each blood blood serum was expressed as the corresponding serum dilution yielding ≥ 90 per cent kill, i.e. the absolute inhibition of bacterial growth defined by the color invariability of the pH indicator, which corresponds to OD in the range of 0.1 to 0.4 at 405 nm of λ max.

Fluorometric Serum Bactericidal Assay (FSBA)

FSBA is nothing but the fluorescent Serum bactericidal assay standardized. Fluorescence is the emission of light from a material that absorbs light or other electromagnetic radiation. Described for the primary time an SBA based on fluorescence (FSBA) that has the ability to interchange other commonly approved SBA configurations. The FSBA described here demonstrates a unique application for the use of alamarBlue in a complement-mediated, antibody-dependent bactericidal assay for the detection of BN. meningitidis. FSBA uses the alamarBlue i.e. Reduction-Oxidation (redox) indicator for the identification of living bacteria after SBA components are permitted to react in microtiter plates. The authors describe alamarBlue as a stable nontoxic, noncarcinogenic, fluorescent, and colorimetric indicator of cellular respiration. Consistent with the manufacturer, the precise indicator incorporated into alamarBlue exhibits both a fluorescence change and a colorimetric change under the acceptable range of cellular metabolic reductions in viable prokaryotic and eukaryotic cells.

Moreover, they suggested that alamarBlue could also be a substitute for molecular oxygen for any of the oxidoreductases within the electron transport system. AlamarBlue has been compared to prokaryotic and eukaryotic cell proliferation indicators like 3-(4,5-dimethylthiazol-2-yl)-2,5-3 acceptable diphenyltetrazolium bromide, 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl]-2H-tetrazolium-5 carboxanilide, and three H thymidine. It has been incorporated into antimicrobial drug susceptibility tests against gram-negative microorganisms and gram-positive microorganisms also as mycobacterium tuberculosis.

The FSBA described during this paper can easily be wont to screen large numbers of individual serum samples in antibody-dependent bactericidal assays. It's relatively easy to perform, and results are generated faster than they are by a colony-counting SBA. FSBA measures the viable count of bacteria between 6 and 8 hrs directly from reaction mixtures in 96-well microtiter plates. Also, colonies isolated on semisolid media could be counted after 24 hours of incubation. The bactericidal titers identified by both assays were nearly equivalent.

The FSBA avoids labour-intensive and tedious measures of plating reaction mixtures and counting the number of individual CFUs that are crucial to understanding the outcomes of typical SBAs. The assay requires direct distinctions to be made between the normal and the inactivated complement.

The FSBA is susceptible method for detection of bactericidal activity serum in patients with gamma globulin or serum deficient for complement factors. The efficacy of FSBA in the quantitative detection of viable organisms is an endearing trait of existing SBA methods in that a more accurate assessment of the serum killing potential can be derived.

Direct fluorescence quantification of the living bacteria eliminates the assay-to-assay variation often found in existing SBA protocols. Plating methods, agglutination of bacteria or dilution errors these types of factors are not affect FSBA. The FSBA also this can be used as an assay for the sampling of large amounts of individual sera as a tool for the identification of functional antibodies directed against group B Neisseria meningitidis in both human and mouse antiserum or supplement sources. The relatively simple performance of

FSBA makes complement screening and evaluation more efficient.

The 96-well microplate format makes FSBA an appealing assay within the sense that it is often adapted to robotic systems almost like those used for ELISA. It's conceivable that during a semiautomated system FSBA throughput would increase and data acquisition and analysis might be standardized.

Conclusion

The investigation for a defensive vaccine against meningococcal serogroups is hindered by a lack of knowledge of the acquisition of natural immunity against this serogroup. The relative contribution to defense of the different isotypes of antibodies and antigen-species detected after systemic infection is not well identified. Further analysis is also required on the relative strengths of specific antigens as protective antigens and thus appropriate for use in vaccines. A candidate vaccine will be thoroughly tested for its immunogenicity and effectiveness. Several laboratory approaches are now available to assess immunity against a variety of meningococcal serogroups. To date, SBA has often been assumed the safest, but not the optimal, process, and many other assay was performed, such as CSBA and FSBA, have been evaluated. Such tend to be sufficient as enhanced safety mechanisms, but it is unclear whether they are more compatible with the effectiveness of the vaccine than with SBA. Both CSBA and FSBA are perhaps the most reflective of the *in vivo* interactions between the immune system and the bacteria, and are thus ideal for large-scale trials. Hence it can be concluded that a mixture of multiple assays is required for assessment of the immune response for post-vaccination in order to determine the consistency of the vaccine candidate. Along with, standardized CSBA will be potentially used to screen number of serum samples, permitting an easy and quick determination of results. This assay will be useful tool for studies, to elucidate the correlates, to detection of functional antibody titer in animal models, vaccine efficiency trials, post-marketing surveillance studies, and epidemiological studies.

References

1. McIntosh EDG, Broker M, Wassil J, et al. Serum bactericidal antibody assays: The role of complement in infection and immunity. Vaccine. 2015;33:4414–21.
2. Stratton CW. Serum Bactericidal Test. Clin Microbiol Rev. 1988;1:19-26.
3. Zent O, Arras-Reiter C, Broeker M, et al. Immediate allergic reactions after vaccinations: A post-marketing surveillance review. Eur J Pediatr. 2002;161:21-5.
4. Bohlke K, Davis RL, Marcy SM, et al. Risk of anaphylaxis after vaccination of children and adolescents. Pediatrics. 2003;112:815-20.
5. Facktor MA, Bernstein RA, Firemann P. Hypersensitivity to tetanus toxoid. J Allergy Clin Immunol. 1973;52:1-12.
6. Cox NH, Moss C, Forsyth A. Allergy to non-toxoid constituents of vaccines and implications for patch testing. Dermatitis. 1988;18:143-46.
7. Vogt T, Landthaler M, Stolz W. Generalized eczema in an 18-month-old boy due to phenoxyethanol in DPT vaccine. Dermatitis. 1998;38:50-1.
8. Miller E, Waight P, Farrington CP, et al. Idiopathic thrombocytopenic purpura and MMR vaccine. Arch Dis Child. 2001;84:227-29.
9. Haber P, Sejvar J, Mikaeloff Y, et al. Vaccines and Guillain-Barre syndrome. Drug Saf. 2009;32:309-23.
10. Kelso JM, Jones RT, Yunginger JW. Anaphylaxis to measles, mumps, and rubella vaccine mediated by IgE to gelatin. J Allergy Clin Immunol. 1993;91:867-72.
11. Sakaguchi M, Nakayama T, Inouye S. Food allergy to gelatin in children with systemic immediate-type reactions, including anaphylaxis, to vaccines. J Allergy Clin Immunol. 1996;98:1058-61.
12. Nakayama T, Aizawa C. Change in gelatin content of vaccines associated with reduction in reports of allergic reactions. J Allergy Clin Immunol. 2000;106:591-92.

*Correspondence to

Dr. Pratik Zagade

Department of Pharmaceutical Chemistry

SGR Sable College of Pharmacy

Pune

India

E-mail: pratikzagade12@gmail.com