UV-Visible spectral detection of vaccinated blood samples of sheep for anthrax

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Abstract: UV-Visible spectroscopic technique is employed to study the spectral differences between pre- and post-vaccinated blood samples of sheep with anthrax spore vaccine. The internal standards among the application peaks were calculated. There was a marked difference in the absorption levels of the pre and post vaccinated blood samples. The resultant variation is attributed to the production of antibodies in the animal. Spectral study can emerge as an alternate and cost effective test for screening vaccinated animal or animal product.

Keywords: Spectroscopy, anthrax spore vaccine, Bacillus anthracis, ELISA test

Introduction

The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years. Blood is the chief circulatory medium in human and in animal body which can be subjected to non-invasive technique for testing. Anthrax, a disease of mammals including human, is caused by a spore-forming bacterium called Bacillus anthracis. Anthrax spore vaccine (ASV) is a glycerinated suspension of live spores of uncapsulated avirulent strain of B. anthracis. Vaccination is the best and cheapest method to protect the body against bacterial and viral diseases. ASV can be used to protect all species of animals viz, cattle, sheep, goat and elephant. Among the various techniques to study the antibody production, ELISA (Enzyme-Linked Immuno Sorbent Assay) is considered to be a better one, which can be done only in sophisticated laboratories.

Evaluation of serologic tests for diagnosis of anthrax after an outbreak was made by Harrison et al (1989). Johnson-Winegar (1984) compared enzyme-linked immunosorbent with indirect hemagglutination assays for determining anthrax antibodies. A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized B. anthracis spore challenge was studied by Mohamed et al (2005). Though many studies have already been carried out on the disease and on the vaccines, no work has been performed using spectroscopic method and the present work aims to employ UV-Visible spectroscopic techniques to analyze the efficacy of Anthrax Spore Vaccine. A spectroscopic method of blood analysis is an alternate technique to the clinical methods since they require fewer samples and provide more information. In this work normal healthy pre vaccinated blood samples (zero day) and post vaccinated (7th, 14th, and 21st day after vaccination) blood samples are analyzed by employing UV-Vis spectroscopic techniques.

Materials and methods

Three healthy ovine female sheep (sheep 1 to 3 each weighing not less than 18 kg), were tested in the Institute of Veterinary Preventive Medicine (IVPM), Ranipet, Vellore District. Eighteen female ovine sheep (numbered 1 to 18) and two male ovine (19&20) sheep were taken in the field level, a village near Kaverippakam, Vellore Dt, Tamilnadu. Blood samples were collected from jugular vein of the sheep. After collecting the blood samples (pre vaccinated or zero day), the animal (sheep 1 to 3) were vaccinated with Anthrax Spore Vaccine (ASV). Blood samples were collected from the same animal on 7th, 14th & 21st day after vaccination. For UV-Vis spectroscopic measurements, the whole blood (citrated blood) was used. The blood samples after dilution with normal saline at a concentration of 0.9% were subjected to spectral analysis using Shimazu UV 1601 Spectrometer in the region 200 to 700 nm. The spectra were taken at Dr. Ceeal Analytical Lab, Chennai, India.

Result and discussion

The UV-Vis spectrum of blood contains information on the absorption and scattering properties of particle suspensions. UV-Vis spectroscopy has been successfully employed in the characterization and conformation of proteins and nucleic acids by many workers. The UV-Vis spectra of all the blood samples exhibit the presence of two strong absorption peaks at 417 nm and 576 nm. But there is a marked difference in the absorption levels of the pre and post vaccinated blood samples. Table 1 summarizes the ratio of the absorption

<table>
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<th>Category</th>
<th>Day</th>
<th>Wavelength and absorbance value</th>
<th>Ratio of the absorbance value A417/A576</th>
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<td>Sheep 1</td>
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<td>8.59</td>
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<td>1.3008 0.2069</td>
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<td>14</td>
<td>0.9234 0.1432</td>
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<td>21</td>
<td>0.8871 0.1346</td>
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<td>21</td>
<td>1.2187 0.1517</td>
<td>8.03</td>
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Table 1. The ratio of absorbance among the peaks using UV-Vis spectra
peaks of the pre and post vaccinated blood samples of sheep, the lab animals at IVPM, Ranipet, Vellore District, Tamilnadu. The ratio of the absorbance value of sheep no 1 was 8.59 and of sheep no 2 & 3, the values were 13.91 and 13.89 respectively in the pre vaccinated state. Blood samples were taken from the same animal on 7th, 14th and 21st day of vaccination. On the 7th day of vaccination the values decreased and on the 14th day it increased slightly. On the 21st day it still increased further. These variations were expected due to be the production of antibodies in the animal between 14th and 21st day of vaccination. Fig. 1 and 2 represent the uv-visible overlaid spectra of sheep no 2 & 3 respectively. The maximum peak corresponding to 417 nm was observed and the overlaid spectra of zero day, 7th day, 14th day and 21st day was shown in figures 1 and 2. The curves were superimposed on each other.

After the 21st day of vaccination the animal were challenged with virulent bacteria (i.e. the live bacteria was injected into the animal body). The antibodies were produced as an anamnestic phenomenon consequent to ‘memory’ established while primary vaccination. After the challenge test, the absorbance ratio of sheep no’s 1, 2 & 3 were 7.12, 8.03 and 7.09 respectively which were greater than that of the 21st day of vaccination due to the production of antibodies.

Table 2 summarizes the ratio of absorbance value of the pre vaccinated blood samples of sheep. The lowest value was 6.78 (sheep no 13) and the highest was 8.83 (sheep no 6). It varies between the two values for the other sheep. These values can be taken as the reference values for the pre vaccinated state of the animal in the field level. In vaccine production centers or institutes, safety and potency test was conducted to test the quality of the vaccine. Animals are generally procured from approved contractors with unknown history. This test can serve to screen animal to be vaccinated and as well to assess the potency of vaccine in vaccine production laboratories.
Animal diseases cause enormous economic loss through mortality, inefficient production and increase in the stock replacement rates, which all require additional resources (Mathur & Dubey, 1994). Control measures in present-day programme include quarantine of imported animals; cooperation of agencies in the study and control of animal diseases; inspection of red meat and poultry to minimize the danger of spread of animal disease to human beings; inspection and evaluation of vaccines and other pharmaceutical and biological products as to purity, efficacy, and safety; inspection of the mass slaughter of animals and the destruction of carcasses. Universities and other research institutions conduct studies on the many disease problems that affect animal of all kinds. Compared to ELISA, UV-Visible spectral analysis is cost-effective test besides it requires small amount of sample for analysis (10 µl). One instrument can analyze infinite number of samples, since it is window based data program-spectrum software. The spectra were baseline corrected and they were normalized to acquire identical area under the curves and the maximum absorbance value of the corresponding characteristics bands was noted. The internal standards among the absorption peaks can be calculated. By studying this, the potency of the vaccine can be assessed.

Conclusion
Spectroscopy has been employed as a diagnostic tool in the study of blood. This spectral analysis can be effectively used as an in vitro test to screen the animal and also assessing potency of vaccine. In vivo challenge test can be avoided once this procedure is standardized which can satisfy the CPCSEA-“Committee for the Purpose of Central and Supervision on Experiments on Animal” - which imposes regulations to use animal for experiments.

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References