2.1 Snakebite epidemiology

Alirol E. et al. (2010) stated that, the actual burden of snakebites remains controversial, although there are many attempts in various parts of the world to find out the actual cases and deaths due to snakebite. According to WHO in India more than 2,00,000 incidences of snakebites occur per year and moreover 35,000 to 50,000 deaths due to snakebites. Gaitonde B.B. et al. (1980) noted that in Maharashtra has the maximum numbers of snakebites, upto 70 bites per one lakh people and deaths are about 2.4 per lakh people/year. National Health profile 2015, published by Central bureau of health intelligence, Ministry of health and family welfare, India estimates as low as about 1,34,980 snakebite cases and 1,180 deaths in the year 2013. The Maharashtra state has about 14,723 cases of snakebites and about 20 deaths due to snakebites. Andhra Pradesh has the highest no. of snakebites about 26680 and about 107 deaths due to snakebites.

It was stated by WHO and many other researchers that the deaths due to snakebites remained unnoticed as in many developing and underdeveloped countries, as the snakebite victims are treated by the traditional healers with the use of herbal medicines and death occurs in their dispensaries. Many die before reaching the hospitals and their deaths remained unnoticed to the authorities remain unnoticed due to improper reporting systems in these countries. In many countries, the data available about the snakebite mortality based on patients admitted to the government hospitals. According to Warrell D.A., (2012), snake venom is a complex mixture of proteins containing hundreds of proteins, which varies from species to species. The variability in proteins depends on the heterogeneity of the species and has diverse biological action on the body during envenomation.

Snake antivenom is the only remedy for snake poisoning. The antivenom is mainly manufactured by hyperimmunization of the equines. The treatment with antivenom requires supportive treatment depends on the venom, whether venom is hemorrhagic, or neurotoxic in nature, and their clinical manifestations. Meenatchisundaran, S. and Michael, A., (2009) reported that snake antivenom is a precious life saving drug having scarcity across the world. As the antivenom contains equine immunoglobulins against the venom, it acts as a foreign protein and have some adverse effects therefore, given to the envenomed patient only. Generally snakebites are
identified by bite marks, general examination of the patient, biochemical tests, 20 min WBCT and immunological methods like radioimmunoassay, immunoelectrophoresis, immunodiffusion, and ELISA. The ELISA is a method of choice for detection of snake envenomationas it has some advantages over the other methods. (J. F. Gao et al., 2013; Selvanayagam and Gopalakrishnakone, 1999; Ibrahim et al., 2013; Casewell et al., 2010; Theakston, 1983; Dong et al., 2004)

2.2 ELISA for snakebite detection

Brunda et al. (2006) used venom specific egg yolk antibodies for detection of Indian cobra venom in biological samples according to him there are many methods for detection of snake venom amongst all these methods ELISA is the ideal method of choice due to its sensitivity and specificity. The use of chick IgY reduces interferences and provides an added advantage in immunoassays. In addition, the use of IgY provided an advantage in reducing the non-specific binding. The time required for the test was about 4 hours.

Dong et al. (2003) developed an ELISA kit for specific detection of venom from four common snakes found in South Vietnam. Lyophilized whole venoms of *C. rhodostoma, N. naja, T. popeorum, and O. hannah* was used for immunization. The IgG antibodies were purified by the protein A affinity chromatography and checked for cross reactivity with heterospecific venoms by ELISA and it was observed by him that antibodies have the cross reactivity with the heterospecific venoms. To remove the cross-reactive antibodies, immunoaffinity chromatography was performed using different venom bound affinity columns. The obtained SSAb’s shown no cross reactivity with heterospecific venom. Further, these antibodies used for preparation of venom detection kits, observed specific and sensitive to detect venoms. The time required for testing was approximately 30 min.

As seen from the above ELISA test is a method of choice due to sensitivity and specificity, but it has some inherent limitation such as, time required is 30 min to four hrs and technical staff required to perform the test. Since snakebites mainly occur in rural areas, facilities to perform the test are scares, also the high cost of the test. Snakebite is an emergency where no doctor can wait until the completion of the test.
2.3 Lateral flow assay

Sajid M. et al. (2014) pointed out in review that recently, more investigations focused on the development of LFA for various types of samples. LFA has lots of advantages over other serological methods like easy test procedure, less sample quantity, rapid results, less expensive, field test, and for analysis of result no expertise required.

Ying Ju et al. (2010) used colloidal gold based LFA for early detection of *Streptococcus suis* serotype 2 (SS2), a gram-positive bacterium responsible for severe infections such as pneumonia, septicemia, meningitis, and sudden death mostly in pigs and humans. For a generation of polyclonal antibodies, rabbits immunized with inactivated, SS2 bacteria. The antibodies were isolated by affinity chromatography and conjugated with 25 nm GNPs. The conjugate was added to the pre-treated conjugate pad and dried at 37° C for 2 h. The test line coated on NC membrane with 2.0 mg/ml rabbit antibody against SS2 and the control line coated with 0.5 mg/ml goat anti-rabbit antibodies. All the components assembled and then cut into 4-mm-wide strips. The results obtained within 5–10 min sample application and the developed LFA specifically detected the analyte of interest.

Literature review demonstrates that venom specific antibodies are utilized in development various ELISA based assays for snake venom detection. LFA has many advantages over other serological methods and used for detection of many bio-molecules using specific antibodies. Considering above points, venom specific antibodies may be used to develop LFA for snake envenomation detection and LFA has potential to become a method of choice, as it is a rapid sensitive and field test.