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Short Communication

Role of bi-specific monoclonal antibodies in immunodiagnostic assay

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This review paper highlights the use of bi-specific monoclonal antibodies (bsMAb) in the diagnostic assays for the early detection of pathogens of human infectious diseases such as severe acute respiratory syndrome (SARS), chikungunya (CHIKV), tuberculosis (TB) and dengue. Bi-specific monoclonal antibodies (bsMAb) are unique and artificially engineered macromolecules with two distinct binding sites, and are capable of binding two different antigens non-covalently. However, the traditional methods of diagnosis such as virus or bacterial isolation, and PCR amplification are quite expensive and time consuming. Bi-specific monoclonal antibodies (bsMAb) are versatile, and can increase the specificity and sensitivity of detection in the suspected individuals. Therefore, immunodiagnostic assays using bsMAb are less expensive, and a large number of clinical samples could be analyzed at a faster rate for the detection of pathogens within a stipulated time. This could allow in developing a cost effective diagnostic kit, which is very useful particularly in the developing countries for the early assessment of the disease outbreak.

Key words: Antibodies, antigen, human-diseases, control-measures, pharmacy, biotechnology

Abbreviations: bsMAB- Bi-specific monoclonal antibodies; BCG- *Bacillus Calmette-Guerin* **CHIKV**- Chikungunya virus ;CoV- coronovirus; DHF-dengue hemorrhagic fever; **DSS**- dengue shock syndrome; **NP**- nucleocapsid protein; **SARS**- severe acute respiratory syndrome; **TB**- tuberculosis

Bi-specific monoclonal antibodies are artificially engineered immunoglobulins with two distinct binding specificities (Suresh *et al.* 1986a, 1986b; Cao and Suresh, 1998). On the other hand monospecific antibodies are the naturally occurring major IgG class having two identical antigen binding paratopes with two identical heavy and light chains (Cao and Suresh, 1998). Bispecific monoclonal antibodies (*bsMAbs*) can be produced mainly by three methods: 1) Chemically linking two antibody molecules, 2) fusion of two different hybridomas and 3) using recombinant DNA approaches. Bispecific mononclonal antibodies have been extensively exploited in both diagnostic and therapeutic areas. Bispecific antibodies were first generated 37 years ago by chemical methods (Nisonoff and Rivers, 1961; Cao and Suresh, 1998). Hybridoma technology opened a wide range of applications in fundamental and applied immunology (Kohler and Milstein, 1975;

Suresh *et al.* 1986a, 1986b). In 1975, a major breakthrough was made by Kohler and Milstein (1975) in generating continuous *in vitro* cultures of fused lymphocytes secreting antibodies with predetermined specificity. Fusion of a normal B cell (plasma cell) with myeloma cell (cancerous plasma cell) resulted in the generation of a hybrid cell known as hybridoma (Kohler and Milstein, 1975; Suresh *et al.* 1986a, 1986b; Cao and Suresh, 1998). This hybridoma not only possessed the immortal-growth properties of the myeloma cell but also secreted an antibody product of the B cells. Kohler and Milstein were awarded a Nobel prize in 1984 for this work (Kohler and Milstein, 1975; Suresh *et al.* 1986a, 1986b; Cao and Suresh, 1998). This landmark discovery has revolutionized the use of bispecific antibodies (*bsMAbs*) because of the specificity of antigen binding abilities. Therefore, *bsMAbs* have many applications in the area of immunodetection, immune-histochemistry, adioimmunotherapy, adio-immunodiagnosis, and immunotherapy to targeted drug delivery, vaccine development and gene therapy (Kohler and Milstein, 1975; Suresh *et al.* 1986a, 1986b; Cao and Suresh, 1998; Khan *et al.* 2011, 2012). Furthermore, *bsMAbs* with intrinsic binding sites to any two antigens has the capability to form uniform, homogeneous and reproducible immunoconjugates with one or two entities in a predetermined order (Suresh *et al.* 1986a, 1986b; Cao and Suresh, 1998). This review paper highlights recent diagnostic applications of bispecific antibodies in immunoswab assay which might play an important role in controlling few of the following human infectious diseases.

1. SARS (severe acute respiratory syndrome).

Severe acute respiratory syndrome (SARS) is a severe form of pneumonia due to Corona virus (SARS-CoV) infection, and has adapted to human to human transmission (Nicholls *et al.* 2003; Das *et al.* 2010; Du *et al.* 2009). The development of specific diagnostic tests against SARS-CoV is an essential step for effective treatment of infected patients and prevents future SARS outbreaks (Das *et al.* 2010). There are three major diagnostic methods are currently available, 1) Viral RNA detection using real time reverse transcription (RT)-PCR (Jiang *et al.* 2004; Poon *et al.* 2004), 2) Antibody detection (Chan *et al.* 2004; Li *et al.* 2005), 3) Antigen detection (Che *et al.* 2004; Li *et al.* 2005). The most predominant SARS-CoV virus derived protein throughout the infection is the nucleocapsid protein (NP) (Hiscox *et al.* 1995).

In our laboratory, five monoclonal antibodies (bsMAbs) against recombinant nucleocapsid protein (NP) of severe acute respiratory syndrome (SARS) causing coronovirus (CoV) were developed by hybridoma technology (Das *et al.* 2010). A highly sensitive, rapid and simple bsMAb-based immunoswab assay was also developed in our laboratory for early detection of SARS-CoV (Kammila *et al.* 2008; Suresh *et al.* 2008; Bhatnagar *et al.* 2008; Das *et al.* 2010). In the immunoswab assay, easy-to-use swabs were first coated with anti-SARS-CoV monoclonal antibody to capture NP in the test sample followed by detection with bsMAb (Kammila *et al.* 2008; Das *et al.* 2010). The immunoswab assay showed NP detection limits of 10 pg/mL (1 pg/swab) in saline, 20–200 pg/mL (1–10 pg/swab) in pig nasopharyngeal aspirate and 500 pg/mL (25 pg/swab) in rabbit serum. This assay was completed within a period of approximately 45 minutes, which make it a rapid test for SARS diagnosis (Kammila *et al.* 2008). In another development, an ultrasensitive ELISA using mouse monoclonal IgG combined with chicken polyclonal IgY antibody for SARS virus infection. The immunoassay method of detecting SARS-CoV NP antigen could be used as a cost effective diagnostic kit for checking the SARS suspected individuals.

2) Dengue

Dengue is one of the viral diseases recorded throughout the world. The mosquito which is responsible for transmission of dengue virus between humans and monkeys is known as *Aedes aegypti* (Murrell *et al.* 2011 Schieffelin *et al.* 2010; Shrivastava *et al*. 2011; Malabadi *et al.* **2010,** 2011; Monath, 1994). Dengue virus is a member of the genus *Flavivirus* (family *Flaviviridae*) (Halstead, 1988, 1989; Murrell *et al.* 2011). In many cases of infected individual, dengue fever (DF) is appeared as the dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Halstead, 1989; Murrell *et al*. 2011). Dengue virus is endemic and significantly causing the death of many children (Murrell *et al*. 2011; Shrivastava *et al*. 2011; WHO, 2009, 2010; Simasathien and Watanaveeradej, 2005; Gubler and Meltzer, 1999). Till today, dengue disease is not under control and effective immunization may provide protection for controlling dengue infections (Murrell *et al*. 2011; Shrivastava *et al.* 2011; Malabadi *et al*. 2011; Schieffelin *et al.* 2010). Therefore, potential vaccine for dengue has been challenging, and there are many problems associated with the development of dengue vaccine (Holmes and Twiddy, 2003; Rico-Hesse, 1990; Murrell *et al.* 2011; Malabadi *et al.* 2011). Long duration immunity to only one serotype is observed in many infected individuals. On the other hand dengue infected individuals showed short duration immunity to the other serotypes (Sabin, 1989; Schieffelin *et al.* 2010; Malabadi *et al.* 2011). One of the major problems is that short duration immunity is lost after 3-4 months, and individual has no immunity to the other three dengue serotypes (Murrell *et al.* 2011; Shrivastava *et al.* 2011).

Currently there is no diagnostic assay available for the detection of dengue virus infection at the acute or early stages. Ganguly and co workers in our laboratory have developed a novel, highly sensitive immunodiagnostic assay for the detection of dengue virus using bsMAb. The assay is highly specific and easy to perform since it does not require any technical expertise or sophisticated instruments. The general format of the immunodiagnostic assay involves coating of dengue virus antigen-specific monoclonal antibody as the capture antibody on calcium alginate tipped swabs with aluminum/or plastic shafts as per previously published protocol (Tang *et al*. 2004).

3) **Chikungunya**

Chikungunya is another viral disease, which was reported for the first time in Tanzania (Robinson, 1955; Muthumani *et al*. 2008). The epidemic cycle of chikungunya is similar to those of dengue and urban yellow fever. Chikungunya virus (CHIKV) is a mosquito-borne alpha virus belongs to the family of *Togaviridae* (Strauss and Strauss, 1994; Tiwari *et al*. 2009; Akahata *et al.* 2010). Geographically, chikungunya virus (CHIKV) is distributed in Africa, India and South East Asia (Mavalankar *et al.* 2007; Tiwari *et al.* 2009). The symptoms of the diseases involves fever, headache, rash, nausea, vomiting, myalgia, and arthralgia/arthritis (Simon *et al.* 2008; Powers and Logue, 2007; Akahata *et al*. 2010). The severity of the disease is characterized by the neurological manifestations such as acute limb weakness, and joint pain with a fatal haemorrhagic condition (Muthumani *et al*. 2008; Mohan *et al.* 2010). Chikungunya was reported as a major epidemic disease during 2004-2007 in Indian Ocean islands and India (Paramasivan *et al*. 2009; Tiwari *et al*. 2009; Mohan *et al*. 2010; Gardner *et al.* 2010). No licensed vaccine or effective drug is available in the market throughout the world for chikungunya disease. However, the expression level of the CHIKV protein is very low in mammalian or bacterial or insect cells. Furthermore, the expressed protein is easily degraded. Therefore, there is an urgent need for the development of diagnostic assay for the early detection of the disease in the infected

individuals. However, current methods of early detection of chikungunya antigen in the infected individuals using PCR amplification are very expensive. The PCR methods involve use of technically trained man power, costly chemicals, and sophisticated instruments for the assay which the developing countries can not afford. Till today, there are no reports of immunoswab assays as the diagnostic approach for chikungunya. Therefore, a sensitive, in expensive diagnostic test using immunoswab assay could be developed for the detection of early stages of the chikungunya viral infection. This immunoswab assay is simple, reliable, fast, and will save of money.

4) Tuberculosis (TB)

The gram positive bacterium which is a causative agent for tuberculosis is known as *Mycobacterium tuberculosis* (Sarkar and Suresh, 2011, Chaudhary *et al.* 2010, Gennaro, 2000). Tuberculosis (TB) is still a major health problem in many countries throughout the world. *M. bovis Bacillus Calmette-Guerin* (BCG) is the only live bacterial vaccine in use that has shown no major side-effects. However, BCG vaccination is ineffective against adult pulmonary TB, and therefore, there is an urgent need for potential vaccines is a high priority. Furthermore, HIV patients and multi-drug resistant mycobacterium strains have contributed to the re-emergence of TB (Sarkar *et al.* 2012). The major problem is that there is no specific diagnostic approach to find out the early stages of TB. Unfortunately, the false negative results of current TB diagnostic test such as *sputum smear microscopy* (SSM) are not reliable (Sarkar *et al*. 2012, Sarkar and Suresh, 2011, Chaudhary *et al*. 2010, Gennaro, 2000). In addition, clinical symptoms and results of chest x-rays are nonspecific (Sarkar *et al.* 2012). PCR based diagnosis is very expensive, which needs costly sophisticated instrumentation and time consuming too. Another simple method is the culture method for the detection of TB. The major drawback of this method is a time consuming since it takes 2-7 weeks to obtain results. Therefore, early diagnosis is very important since TB is a highly contagious disease at the early stages of development. Therefore, our laboratory has developed a novel, and highly sensitive immunodiagnostic assay for the detection of *M. tuberculosis* using *bsMAb* (Sarkar *et al.* 2012). TB diagnostic test was developed as per Sarkar *et al.* (2012). The assay is highly specific, sensitive, very fast to get results, and very less expensive.

Therefore, the use of *bsMAb* in the immunodiagnostic assays plays an important role in the modern medicine. This method of diagnostic approach is very simple, rapid and cost effective. Early diagnosis always helps the patients in controlling the spread of disease at the right time. It will also help the physicians for recommending the proper medication for controlling the disease in the infected individual.

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