CHAPTER 1
DEVELOPMENT OF MONOCLONAL ANTIBODY BASED IMMUNODIAGNOSTIC TESTS FOR ADENOVIRUSES CAUSING RESPIRATORY INFECTIONS

INTRODUCTION

Adenovirus infections are distributed worldwide. These viruses are associated with a variety of disease syndromes like upper and lower respiratory illness, pertussis, acute respiratory infections, keratoconjunctivitis, pharyngo-conjunctivitis fever, acute haemorrhagic conjunctivitis, gastroenteritis, central nervous system infections and sexually transmitted diseases. The incidence and severity of these infections are increasingly observed in immunocompromised hosts with case fatality rates as high as 60 per cent in those with pneumonia and 50 per cent in those with hepatitis (Hierholzer, 1992).

The most unusual aspects of the adenoviruses isolated from AIDS patients are the diversity of serotypes involved and the high frequency of isolation of antigenically intermediate strains. The antigenically intermediate strains are important because their stay in the AIDS patients presumably extends from the time of onset of adenovirus infection to death of the patient. This long term persistence can lead to the mutations in the strain or may allow recombinational events between coinfecting serotypes, resulting in the appearance new antigenic strains (Hierholzer, 1992).
The most common syndrome associated with adenoviruses involves the respiratory tract (Brandt et al., 1969; Knight and Kasel, 1973). Extensive epidemiological surveys have placed the adenoviral agents as the cause of 5 to 10 per cent of total juvenile pneumonia and respiratory infections of north American citizens (Fox et al., 1969; Brandt et al., 1972; Foy et al., 1973). In infants and young children, 10 per cent of all respiratory infections are caused by these organisms with mortality in number of infants and considerable morbidity in certain adult population (Mogabgab, 1968; Brandt et al., 1969; Simila et al., 1971; Knight and Kasel 1973). Many of these infections are relatively asymptomatic, whereas a significant proportion of the remainder takes place in the form of coryza, mild pharyngitis or tracheitis. Although a bacterium *Bordetella pertussis* is the cause of true whooping cough, adenovirus has been isolated from many patients with this syndrome, either in conjunction with the *B. pertussis* infection or alone (Olson, 1975). In young adults, especially those who live in close communities such as military recruit camps and boarding schools, adenovirus may also cause epidemics of influenza like illness, including tracheobronchitis and pneumonia severe enough to warrant hospitalization. The incubation period of the disease is four to five days, and cough, rhinorrhea, fever and sore throat, the most common symptoms, last for another three to five days (Hillemann and Werner, 1954; Simila et al., 1971). In this adult population, adenoviruses causes upto 40 per cent of the atypical pneumonia or the adult respiratory disease syndrome, the remainder being caused by *Mycoplasma pneumoniae*, *Legionella pneumophila*, influenza and para-influenza viruses and other viral agents. The infection is self limited, there is no specific therapy and superinfection and deaths are not common (Hillemann et al., 1954). Central nervous system can be involved
following adenovirus respiratory infections, either sporadically or as epidemic meningoencephalitis and encephalitis (Hierholzer, 1989; Horwitz, 1990).

Indian reports on the involvement of adenoviruses in disease syndromes are scanty. An earlier work carried out in West Bengal revealed a remarkably high infection rate with pronounced seasonality in children aged from newborns to five years (Kloene et al., 1970; Hill et al., 1973).

Adenoviruses are non-enveloped, double-stranded DNA viruses with a size of 70-90 nm in diameter. The genome has molecular weight of about 23 x 10⁶ and G+C content of 48-61 per cent. The DNA is associated with several core proteins and is enclosed in a protein shell called capsid with a icosahedron symmetry. The capsid contains two types of capsomeres, hexons and pentons. Each penton consists of a base and a fibre. The capsid protects the DNA from physical and chemical degradation. The fibre sub units help the virus for its attachment to host cell surface receptors (Norrby, 1969). It is a predominant type specific protein which as a part of penton component comprises the 12 capsomeres at the vertices of the adenoviruses. It is present in excess but is labile in nature and is poorly immunogenic. The principle group specific antigen is hexon, which is a highly stable protein comprising 240 of the 252 capsomeres of the adenovirion. In addition, the hexon is produced in prodigious excess during viral replication and is highly immunogenic. Thus this component forms the basis of group reactive assay systems used in certain laboratories to diagnose adenoviral infections (Norrby, 1969; Wadell, 1988; Horwitz, 1990; Hierholzer, 1991).
In the ensuing years, 47 serotypes of human adenovirus has been isolated (Norrby, 1969; Wadell, 1988; Horwitz, 1990). Several classification schemes have been developed on the basis of characteristics of the pathogen. These schemes have focused on the ability of certain adenoviruses to agglutinate erythrocytes of different animal species (Rosen, 1958); serological differences in surface antigens (Mathews, 1981); the ability to cause tumours in animals and transform tissue culture cells (Freeman et al., 1967; Ginsberg, 1980); and guanine-plus-cytosine (G+C) content of specific adenovirus genome (Green et al., 1967).

Most often, diagnosis of adenovirus infection rests on clinical criteria in the appropriate epidemiological setting. Confirmation usually involves isolation and identification of virus or rise in antibody titre during patient's convalescent period. Immuno-electron microscopy is useful, if available, in diagnosing adenovirus diarrhoea. The virus can be isolated relatively easily from the appropriate body fluids, respiratory tract or conjunctival secretions by culture on human epithelial cells. Pathognomonic cytopathic effect (CPE) takes place in three to seven days, depending on the amount of virus in the inoculum. Identification of adenovirus is then carried out by haemagglutination grouping, followed by serotyping using complement fixation, neutralization, haemagglutination inhibition or enzyme immuno-assay techniques. Immunofluorescence assays of exfoliated infected cells have also been used for the diagnosis (Schwartz et al., 1976). More recently monoclonal antibody based fluorescent assay, latex agglutination and enzyme linked immuno-sorbent assay (ELISA) and DNA hybridization techniques have been used for virus identification. These techniques are of particular importance in diagnosis of enteric adenoviruses, where conventional cell culture
techniques fail (Gardner and Quillin, 1974; Herrmann et al., 1987). Reliable immunodiagnostic tests for the identification of adenoviruses in respiratory illness, however, are not available in our country. The present study proposes to develop specific monoclonal antibodies against adenoviruses causing respiratory infections in human beings and to utilize them in developing immuno-detection tests. Applications are also proposed on clinical specimens of acute respiratory disease patients along with comparison of conventional cell culture isolation and identification techniques.