CHAPTER I

INTRODUCTION

While numerous method of determining circulating antibody are available, virtually all tests that have found widespread application require rather large quantity of serum, which usually obtained by venipuncture.

Now a new technique, namely hemagglutination-inhibition micromethod has been developed. This technique requires only a small amount of serum. Previous workers usually collected the blood by venipuncture which is difficult to do with young children, and not practical for the regions where the transportation is difficult and lack of laboratory facilities. In order to solve this problem, many investigators have been trying to modify the technique for collecting the small volume of blood.

From time to time, many researchers have been trying to modify the technique for antibody determination. It is obvious therefore, to consider the use of filter paper as a transportmedium for blood or serum, this would largely correct the drawbacks. Blood or serum dried on filter paper may be despatched by post. Blood samples may be obtained simply by pricking a finger or ear. This filter paper method eliminates the use of glassware in the field and offers certain advantages in survey work and greatly facilitates collection of blood specimens, especially from infants.

Since 1939, filter paper had been used as a transportmedium for blood samples in the serological examination of bacterial and virus infections. Zimmermann (32) and Demanche (32) used this filter paper method for syphilis

serology. Brede (32), Wolff, Heirman and Bohlander (32) and Van Thiel, Van der Hoeven and Couve (44) used filter paper in leptospirosis research. Wolff (32) and Oppelaar (32) demonstrated the suitability of filter paper in the investigation of Salmonella antibodies. Vaisman, Hamelin and Guthe (43) used fluorescent antibody technique to detect antibody against treponema in the blood eluted from blotting paper. Adams and Hanson (1) described the method of drying serum samples on absorbent paper for the study of antibodies against vesicular stomatitis. Karstad, Spalatin and Hanson (29) indicated that the paper disc method for the procurement and handling of blood and serum samples can be used in the serological survey for antibodies against eastern equine encephalomyelitis virus. Kalter (27), De Sommer and Prinzie (19) and Farrell and Reid (21) have conducted assays for poliovirus antibodies by using paper discs moistened with serum and applied to the surface of agar-overlaid infected tissue cultures. Green and Opton (23) described the technique for measuring poliovirus antibody which requires a small amount of whole blood collected from a lancet puncture by absorption on filter paper. Anderson, et al. (2) and Sadun, et al. (37) utilized blood or serum collected on filter paper in the serodiagnosis of schistosomiasis by fluorescent antibody techniques. Worth (49) used filter paper in mumps research. Brody (6) used in hemagglutination-inhibition and neutralization reactions and demonstrated the usefulness of whole blood collected on filter paper discs and store in the dry state for routine HA-test for arboviruses. Kalter (28) used serum-impregnated filter-paper discs rather than serum collected by venipuncture in identification and titration of cytopathic viruses and detection of antibodies by neutralization test. Worth (49) reported the standardization and successful use of the filter paper method of blood collection in mumps HAI antibody. Brody, et al. (7) showed that the use of dried blood collected on filter paper discs was feasible in routine complement-fixation

tests for adenoviruses and in hemagglutination-inhibition tests for measles. Gaggero and Sutmoller (22) used serum and blood dried on blotting paper in the detection of foot-and-mouth disease antibody. Dighero, et al. (20) described the preparation of antisera to human mycoplasma and their use when dried on paper discs.

Different types of filter paper were used and recommended by different investigators. Vaisman, et al. (43) reported that only blotting paper, type Canson 435, were usable but electrophoresis paper and membrane filters were unsuitable. Oppelaar (32) used blotting paper, type Canson 435, and 3 types of filter paper namely Delta 310, Schleicher and Schull 589 (ss)and Whatman 1. Brody (6) used filter paper discs of the type used in antibiotic-sensitivity tests.

Some investigators used filter paper to absorb serum instead of absorb blood. Karstad, et al. (29) found that serum against eastern equine encephalomyelitis virus absorbed on filter paper was less satisfactory than absorbed blood but some investigators preferred serum to blood. This contradiction due to different methods for detecting antibody. The use of serum on filter paper should be restricted to cases in which dried blood samples cannot be used in the laboratory for technical reasons. This may be the case in serological reactions with low titers in which the hemolytic eluate of the blood discs impedes the reading, as recorded by Karstad, et al. (29) in their investigations with the complement fixation reaction.

Dengue infection (breakbone fever, dandy fever, sun fever or solar fever) which will be detected in this study is caused by filterable virus, namely dengue virus. This virus is transmitted by mosquitoes, Aedes aegypti and Aedes albopictus. Characteristic clinical features were eruptive and febrile disease, coming on suddenly, and marked by severe pains in the head, eyes, muscles, and joints, sore throat, catarrhal symptoms, and sometimes a cutaneous eruption and painful

swellings of the parts. The disease comes on suddenly after an incubation period of from three to six days. The symptoms increase in severity for two or three days, then decrease somewhat, only to increase again on the fourth or fifth day, at which time the eruption appears.

Dengue virus is in arbovirus group B. It is RNA, enveloped virus with diameter about 17-25 mμ. Within group B there are certain complexes of viruses which are especially closely related to one another immunologically and which also tend to have certain biologic properties in common. There is one aspect of the viruses of group B which can be a serious problem to both the clinician and the epidemiologist. The degree of antigenic cross-reactivity is much more marked in group B than in group A and may make specific serologic diagnosis of current or past infections difficult or impossible. As a general rule, the most rapid and definitive method of diagnosis of current infections is by virus isolation; however, this is often impossible and impractical. Therefore, most diagnoses must rest on serologic evidence.(35, 48).

Many viruses possess the capacity to agglutinate the erythrocytes of certain mammalian or avain species. Viral antibody may thus be assayed for its ability to specifically inhibit hemagglutination, since the combination of antibody with virus prevents the hemagglutinins from attaching to receptors on the surface of erythrocytes; the assay technique based on this property is called the hemagglutination-inhibition test. Certain nonspecific substances present in sera and other biologic fluids may also prevent hemagglutination, and they must be removed prior to examination for the presence of specific hemagglutination-inhibiting (HI) antibodies. (8, 39) This study will use the hemagglutination-inhibition test for the detection of dengue HI-antibody.

Plan of Investigation

The following studies include:

(i) The determination of the types of immunoglobulins eluted from filter paper strips and compare those with serum collected by syringe.

(ii) The comparison of the titer of antibody eluted from filter paper strips and from syringe.

(iii) Effect of environmental factors on the titer of antibody absorbed on filter paper strips.