Use of Serum Stored on Filter Paper Disks in Complement Fixation Tests for Adenovirus Antibody

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A simple method of storage and retrieval of serum samples for adenovirus serology is described. Complement-fixing antibodies remained stable for at least 5 years when stored at -10°C or below.

A simple method for storage and retrieval of serum samples for serological studies has yet to be described. This is particularly true for those agencies that maintain large banks of serum samples which are used for epidemiological studies by several institutional or international investigators. The most common method of storing sera has been in the frozen state at -10 or -20°C . Unless several portions of each serum are made, the frequent freezing-thawing that takes place in sample retrieval over many months or years may lead to possible contamination of the sample and denaturation of the serum proteins. It has been reported that blood can be collected on filter paper disks and transported as a dried sample to a laboratory facility for serology testing (1-6, 9). For the studies described in this report, sera were dried on filter paper disks (Carl Schleicher & Schuell Co., Keene, N.H.; no. 740-E, 0.5-inch [ca. 1.27-cm] diameter) and stored at different temperatures to be tested periodically over a 2-year period and again after 5 years for those disks stored at -10 and -70°C to determine the stability of serum antibody against a single agent, the adenovirus. The sera of 10 individuals were preserved by pipetting 0.1 ml of each serum onto each of four sets of filter paper disks, each set having 10 disks. The disks were placed on a simply made hail screen rack (8 by 10 inches [ca. 20.3 by 25.4 cm]) for loading. They were dried at 37°C for 1 h and stored in a 1-dram (ca. 1.18-g) vial. One set each was stored at room temperature and at 6, -10 , and -70°C . In addition, 10 0.5-ml samples of each serum were dispensed and stored at -10°C until tested. Each test day, a disk was removed from the vial with forceps and placed in a test tube (13 by 75 mm) with 0.4 ml of 0.15 M NaCl (saline). The disk was stirred in the saline by a Vortex mixer for 5 to 10 s, allowed to stand at 6°C overnight, and stirred again for 5 s. The eluate was heated at 56°C for 30 min and tested for complement-fixing (CF) antibody as previously described

(7). Primary incubation was for 18 h at 6°C . The data in Table 1 show the mean titer of the 10 sera that had been stored at different temperatures over varying lengths of time. Sera stored on filter paper for a 30-day period, regardless of temperature, gave titers equivalent to those of the fresh sera. However, by 6 months an average of approximately one-third of the original titer was lost in those samples stored at room temperature, and by 12 months an average of two-thirds of the original titer was lost. Because of this loss in antibody titer level, samples stored at room temperature were not tested further. In contrast, titers of those samples stored at either refrigeration temperature or at -10 to -70°C gave titers equivalent to those of sera stored at -10°C over the 2-year testing period. Sera stored on filter paper at either -10 or -70°C gave equivalent titers to the fresh sera when stored as long as 5 years.

These data confirm and extend previously reported results (2, 3, 5, 9) showing that serum viral antibody activity is stable in sera stored or transported after drying on filter paper. Brody (1) showed that hemagglutination inhibition and neutralization antibodies to several arboviral agents were stable for at least 12 months when whole blood was dried on filter paper disks; the storage temperature was -20°C . The results presented here would indicate that CF antibody (at least to the adenovirus) is stable for at least 5 years after serum had been dried on filter paper. A preliminary study, yet incomplete, indicates that sera stored for as long as 8 years on filter paper at -10 or -70°C give CF and neutralization antibody titers to adenovirus type 4 comparable to those of the same sera stored in the frozen state at -10°C . It should be emphasized that not all classes of immunoglobulins are satisfactorily preserved by drying on filter paper (3, 8). Studies would have to be performed on each antigen-antibody test system before utilizing this method of serum antibody preservation.

TABLE 1. Geometric mean CF antibody titer of 10 sera stored at different temperatures for varying time periods

Storage temp	Geometric mean CF antibody titer								
	24 h	5 days	12 days	30 days	60 days	12 months	18 months	24 months	60 months
Sera ^a (-10°C)	34.0	32.0	34.3	29.9	32.0	34.3	34.3	32.0	32.0
Filter disks									
Room temp	34.3	34.0	32.0	29.9	21.4	11.5	NT ^b	NT	NT
Refrigeration temp	36.8	34.3	32.0	32.0	29.9	32.0	34.3	29.9	NT
-10°C	34.3	36.8	32.0	34.3	32.0	36.8	34.3	32.0	32.0
-70°C	36.8	34.3	34.3	29.9	34.3	34.3	32.0	29.9	34.3

^a A serum sample from each subject was tested the same day as the eluates from filter paper. Sera were stored in 0.5-ml volumes at -10°C.

^b NT, Not tested.

Information is lacking on the stability of disk-stored serum antibody at room temperature after storage for variable periods of time at 6°C or below. This would be important in instances where sera were mailed to another laboratory facility, and a delay of a week or longer might occur before testing could be accomplished. Ability to mail paper disks in an ordinary envelope would simplify epidemiological studies as compared with the handling/mailing of frozen sera.

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