



THE PRESERVATION OF STANDARD SERA FOR SEROLOGY OF
 PARASITIC DISEASES

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1. INTRODUCTION

It is essential in any serology laboratory to be able to store a bank of antisera for use as controls in day-to-day routine tests, as well as for titration and evaluation of new antigens and techniques. Ideally, it should be possible to store these controls for long periods without loss of titre, and they should be easily accessible. Current methods of storage are normally by refrigeration: in deep freezers at -20°C to -70°C , in specialized dry-ice boxes at -80°C or in liquid nitrogen containers at -196°C , all of which have some disadvantages. None is convenient for transport. Freeze-drying provides an alternative to low temperature preservation but it is not widely used. This paper presents data on various aspects of storage and transportation.

2. METHODS

2.1 Sera

All sera were freshly obtained under aseptic conditions. No preservatives were added. They were either known positive controls for filariasis or schistosomiasis or were being tested for antibodies against these diseases. They were tested by a complement fixation (CF) test, the technique being a modification of the LBCF method (United States Public Health Service, 1965).

2.2 Liquid nitrogen storage

Samples were always frozen quickly by plunging them directly into the liquid. Prior to use they were thawed rapidly in a waterbath at 56°C .

2.3 Freeze-drying

(a) This was carried out using an Edwards centrifugal freeze-dryer (Model 5PS). Twelve sera were tested for the effect of freeze-drying without storage. These were dried, left unsealed at $+4^{\circ}\text{C}$ overnight and tested the next day. After initial drying the samples of two sera for long-term testing were left overnight over a desiccant and then sealed under vacuum in glass ampoules. They were subsequently stored at room temperature. Before testing, samples were reconstituted with distilled water to their original volume.

(b) Thirty-nine sera from patients with clinically confirmed schistosomiasis were received freeze-dried and in duplicate from Africa. One of each pair was reconstituted and tested on arrival and then stored in liquid nitrogen. The other was stored in the dried state at -20°C . These were tested three months later in parallel.

2.4 Drying on filter-paper

In volumes of 0.2 ml, sera were absorbed on discs of Whatman No. 1 filter-paper. These were allowed to dry under atmospheric conditions and then were stored over calcium chloride or in plastic or paper envelopes for three to four weeks at room temperature. They were eluted in 2 ml of veronal buffered saline pH 7.2.

3. RESULTS

3.1 Effect of temperature on storage of serum

Table 1 shows the effect of storage on aliquots of a pool of known positive sera at different temperatures. Results with the CF test are expressed as either negative or the reciprocal of the antibody titre giving 50 per cent. CF. Each aliquot was tested once only and the remainder discarded, thus precluding possible interference by repeated freezing and thawing. The results show that control antisera can be kept at 4°C for up to six weeks, at -20°C for three

months and at -196°C for almost four years without a significant drop in titre. However we have noticed that samples of serum removed from the top of a cane have shown a drop in antibody titre over a period of years whereas the samples of the same serum at the bottom of the cane had retained their titre.

3.2 Effect of freezing and thawing

The effect of repeated freezing and thawing of sera is shown in Table 2. Aliquots of a pool of fresh sera were stored at either -20°C or -196°C . They were thawed from one to 12 times and were allowed to remain thawed for either one-half hour or overnight. Samples were kept frozen for at least 24 hours between each thawing. The experiment was conducted over a period of five weeks, during which time no deterioration due to storage should be apparent. Loss of titre occurred in all cases but was least marked when the time spent unfrozen was short and storage was at -196°C . This conclusion was supported by a further experiment in which a serum sample was thawed for the removal of a single aliquot and refrozen immediately. In this case there was no loss of antibody titre throughout 10 cycles of freezing and thawing.

The effect of repeated freezing and thawing was also studied in a batch of sera which had previously been stored for three years at -196°C . These all showed a deterioration in antibody titre, but this was no more marked than in the fresh serum.

3.3 Preservation by freeze-drying

Four of the 12 sera tested immediately after freeze-drying showed a drop in antibody titre of one tube. Eight showed no change. Preliminary results on the preservation of sera by freeze-drying compared with the same sera stored at -196°C are given in Table 3. Over an eight-month period there was no significant loss of titre in either case.

Of the sera received from Africa 28 out of 39 gave positive reactions on arrival. After three months those stored in nitrogen comprised 17 positives, 18 negatives and four were anticomplementary. Those that had remained freeze-dried comprised 20 positives, 18 negatives and one anticomplementary. Among the negative sera those that had originally been positive had all given weak reactions (5+ or 10+) initially.

3.4 Preservation by drying on filter-paper

Table 4 shows the effect of storage by this method over a period of four weeks, the samples being stored in a desiccator over calcium chloride. There is a significant deterioration in antibody titre. The effect of storage in sealed plastic and in paper envelopes was also compared. The deterioration of the samples in the plastic envelopes over a three-week period was similar to that of the samples in the desiccator. But the fall of titre was more marked in those stored in the paper envelopes which were permeable. If the sera were eluted from the filter-paper immediately after drying, there was no drop in antibody titre. Therefore it would appear that the action of drying does not affect the titre, but subsequent degradation occurs which can be reduced by storage under dry conditions. Of 12 sera that were sent to us from Ceylon for filaria CF test, dried and sealed in plastic envelopes, eight were negative, three positive and one anticomplementary.

4. DISCUSSION

The problems encountered in maintaining a serum bank and transporting control or test sera between laboratories have three causes:

- (1) serum cannot be preserved for long periods by refrigeration unless the temperature is held below the eutectic point of -60°C (Greaves, 1954);
- (2) methods of storage at very low temperatures are somewhat cumbersome and expensive;

(3) since repeated freezing and thawing cause loss of titre, it is desirable that sera should be stored as aliquot samples suitable for individual testing. The limited capacity of a liquid nitrogen container makes it unsuitable in this respect.

These problems are common knowledge but not much appears to have been written about them, and it is still the practice in many laboratories to store sera at -20°C .

The results obtained in this study confirm that sera cannot be stored without serious loss of CF titre except at very low temperatures, though an ordinary refrigerator is suitable for periods up to four weeks. A deep freezer working at -20°C is not very much better. Results with sera stored in liquid nitrogen were excellent for periods up to three-and-one-half years but we gained an impression that sera at the top of the container are apt to deteriorate if the liquid is allowed to sink low and the container is opened fairly frequently. This means that the nitrogen supply must be topped up at short intervals, which is inconvenient. Another finding was that, if sera are thawed and have to be refrozen, it is important that they should be refrozen without delay. If there is no delay, there is virtually no deterioration.

Freeze-drying of sera would appear to be the method of choice for many purposes. Provided they are sealed under vacuum or in a state of complete desiccation they can be stored at room temperature for considerable periods without deterioration. Further investigation of this method is needed.

The experience of previous workers (Pellegrino & Brener, 1958) is confirmed: sera dried on blotting-paper can be sent through the post for CF testing. But unless they are kept in a state of desiccation, deterioration is rapid with loss of specific potency and development of an anticomplementary property. Nevertheless this method is superior to the sending of sera in the fresh state in a hot climate.

TABLE 1. THE EFFECT OF STORAGE AT DIFFERENT TEMPERATURES ON TITRES OF SERA

| Period | Temperature | | | |
|--------------|-------------|----------|----------|--------|
| | 37°C | 4°C | -20°C | -196°C |
| 0 | 160 | 160 | 160 | 160 |
| 1/2 week | 160 | * | * | 160 |
| 1 week | 40 | * | * | 160 |
| 1-1/2 weeks | 40 | * | * | 160 |
| 2 weeks | 10 | * | * | 160 |
| 2-1/2 weeks | 5 | 160 | * | 160 |
| 4 weeks | Negative | 80 | * | 160 |
| 6-1/2 weeks | | 20 | * | 160 |
| 9-1/2 weeks | | Negative | 40 | 160 |
| 11-1/2 weeks | | | 20 | * |
| 6 months | | | Negative | 160 |
| 10 months | | | | 160 |
| 3-1/2 years | | | | 160 |

* Not tested.

TABLE 2. THE EFFECT OF REPEATED FREEZING AND THAWING

| Titres* of sera stored in liquid nitrogen | | | Titres* of sera stored at -20°C | | |
|--|------------------|-----------|------------------------------------|------------------|-----------|
| No. of thaws | Time left thawed | | No. of thaws | Time left thawed | |
| | 1/2 hour | Overnight | | 1/2 hour | Overnight |
| 1 | 80 | 80 | 1 | 40 | 40 |
| 2 | 80 | 40 | 2 | 40 | 20 |
| 3 | 80 | 20 | 3 | 20 | 40 |
| 4 | 80 | 20 | 4 | 20 | 20 |
| 5 | 80 | 20 | 5 | 20 | 20 |
| 6 | 80 | 20 | 6 | 20 | 20 |
| 7 | 40 | 20 | 7 | 20 | 20 |
| 8 | 20 | 20 | 8 | 20 | 20 |
| 9 | 20 | 20 | 9 | 10 | 20 |
| 10 | 20 | 20 | 10 | 10 | 20 |
| 11 | 20 | 20 | 11 | | |
| 12 | 20 | 20 | 12 | | |

* Original titre 80.

TABLE 3. THE EFFECT OF FREEZE-DRYING ON TITRES OF SERA SEALED UNDER VACUUM WITH SUBSEQUENT STORAGE AT ROOM TEMPERATURE

| Period | Titres of freeze-dried sera | | Titres of sera at -196°C | |
|----------|--------------------------------|------------|-----------------------------|------------|
| | Serum A | Serum B | Serum A | Serum B |
| 0 | 40 | 20 | 40 | 20 |
| 4 months | 20 | 20 | 40 | 20 |
| 8 months | 20 | 20 | 20 | 20 |

TABLE 4. FALL OF TITRES OF SERA DRIED ON FILTER-PAPER AND STORED IN A DESICCATOR

| Serum tested | Titre on drying | Titre after 4 weeks |
|--------------|-----------------|---------------------|
| 1 | 160 | 40 |
| 2 | 80 | 40 |
| 3 | 80 | 40 |
| 4 | 5 | 5 |
| 5 | - | - |
| 6 | 20 | 10 |
| 7 | 40 | 10 |
| 8 | 20 | 10 |
| 9 | 80 | 40 |
| 10 | 10 | 5 |

SUMMARY

The problem of storage of control sera for immunological tests has been investigated with the following results.

Unpreserved sera were kept without serious loss of CF titre for four weeks at 4°C, three months at -20°C, and three-and-one-half years (or more) at -196°C.

Sera could be thawed approximately seven times without serious loss of titre provided refreezing was carried out within one-half hour; but only two or three times if the sera were left unfrozen for 18 hours.

Lyophilization was a promising method of storage and transport.

Sera dried on filter-paper deteriorated rapidly, but the method was of some value for sending by post, though not for storage.

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