The cryopreservation of standard sera

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SUMMARY To determine the storage temperature needed for the preservation of standard sera for serodiagnostic tests, groups of sera were tested before and after storage at -70°C and -20°C for periods of up to five years. The temperature required depended on the nature of the test, but no definite advantage was found in storage at -70°C . For complement fixation this temperature was inadequate and greatly inferior to liquid nitrogen. For immunofluorescence it had no apparent advantage over a temperature of -20°C , and for some other tests it was no more than marginally superior.

Despite the extensive literature on the cryopreservation of blood cells, tissues, and organisms, reviewed by Pegg,¹ there appears to have been very little work on the storage of standard and reference sera, which are an essential adjunct for any serodiagnostic programme. Although there may have been other reports on the subject the only one we have come across since Greaves² account of 1954 is the paper from this department, in which Woodhead³ found that a temperature of -20°C was unsuitable for the purpose of complement fixation tests even for relatively short periods. Although liquid nitrogen is generally recognised to be the ideal medium for storage, because of its inconvenience and expense it remains common practice to store sera in domestic freezers which operate at about -20°C , a temperature at which the salt concentration in the unfrozen component of the serum would be extremely high (in the case of saline about 5 M).¹ Freezers that operate below the eutectic point of serum (-60°C^2), at which freezing is finally complete, are relatively cumbersome and expensive. And although many cells and tissues can be stored successfully at -70° to -80°C this often depends on the use of a cryoprotective agent, such as glycerol.¹

We report the effect of different serum storage temperatures on various serodiagnostic procedures after periods of up to five years.

Methods

Sera were available from patients referred to this hospital for the investigation of possible parasitic

disease. Serum antibody levels were tested by a standard indirect immunofluorescence (IF) technique for filariasis and amoebiasis, complement fixation (CF) for schistosomiasis,⁴ and cellulose acetate precipitation (CAP)⁵ and counter-immunoelectrophoresis (CIEP)⁶ for amoebiasis. The antibody titre of sera tested by IF and CF was noted, whereas CAP and CIEP were recorded only as positive or negative. The routine procedure was to store test sera at 4°C for periods of one to seven days before testing. Those sera that had been found to have positive antibody levels were subsequently placed in small aliquots at -20°C or -70°C . Only those sera tested by CF had been heat-inactivated, and no preservatives were added to the sera before storage. The date of storage was recorded on each individual aliquot.

After a known period of storage of one to five years all the stored sera were retested, and any fall in titre by CF or IF, or loss of positivity by CAP or CIEP, was noted. The source of supply and method of preparation of all antigens remained constant throughout the study.

Results

As far as could be judged from the limited range of antigens employed, all of either protozoal or helminthic origin, the nature of the antigen did not affect the outcome of the investigation. However, the effect of storage temperature on the deterioration of serum varied considerably, depending on the type of serological test (Table).

The sera stored at -70°C and tested by CF deteriorated rapidly with time. After three years some

Effect of storage temperature on deterioration of serum

Test	No. of sera	Storage temperature (°C)	Period of storage (yr)	% showing fall in titre	% conversion to negativity
CF	4	-70	5	100	50
	10		4	80	50
	10		3	50	20
	10		2	30	0
	10		1	10	0
IF	4	-70	5	0	0
	10		4	0	0
	10		3	0	0
	10		2	0	0
	10		1	0	0
	10	-20	3	0	0
	10		2	0	0
	10		1	0	0
	10		1	0	0
	10		1	0	0
CIEP	10	-70	1	...	0
	20	-20	1	...	10
CAP	10	-70	1	...	0
	10	-20	1	...	10

of the sera showed a fourfold drop in titre. After five years all showed a fall of at least one or two doubling dilutions.

Sera tested by CIEP and CAP had been stored for only a limited period at the time of retesting. There was some loss of positivity of sera stored at -20°C but no apparent deterioration of sera stored at -70°C. However, during this period the sera stored at -20°C were inadvertently thawed owing to failure of equipment for two periods of about 12 hours.

Sera tested by IF for both filariasis and amoebiasis showed no loss of titre over a period of five years, either at -70°C or at -20°C, despite thawing at -20°C.

Discussion

The conclusion of this study is that there is little advantage in storing sera at -70°C, which requires fairly cumbersome and expensive equipment. For CF testing -70°C was inadequate, except for short periods, and greatly inferior to liquid nitrogen.³ For IF a domestic freezer was adequate for periods of up to five years. For CIEP and CAP -70°C might have had some advantage over -20°C, though the apparent disadvantage of the latter could have been due to accidental thawing, even though this did not affect the IF results.

It seems clear that the antibodies involved in these tests are heat labile, in descending order from CF to CAP or CIEP to IF, which is apparently stable. A possible explanation for these differences may be that different serological techniques detect

different classes of immunoglobulin, and it is these immunoglobulin classes that differ in their stability at low temperatures. Under the conditions of routine tests IF is known to measure mainly IgG and CF mainly IgM, which on this hypothesis implies that IgM might be more labile than IgG. The nature of the antibody involved in CAP and CIEP tests is not definitely known, but there is at least a suggestion that CAP might be dependent on IgG rather than on IgM.⁷

References

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