EVALUATION OF VARIOUS PRESERVATIVES FOR V.D.R.L. ANTIGEN

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Summary

V. D. R. L. antigen once reconstituted has to be used the same day. To prevent wastage in the case of a possible delay in performing the test, the reconstituted antigen was preserved at lower temperatures like 4°C and 0°C and also by certain reducing agents like cystein, merthiolate, formaldehyde, thioglycollate, benzoic acid and potassium metabisulfite at the final concentrations of 0.05%, 0.001%, 0.05%, 0.05%, 0.02% and 10 parts per million respectively. The reactivity of these antigens was tested at regular intervals with known positive sera. It is rewarding to note that most of the methods could preserve the antigen for 17 days to 42 days. The best of all being freezing at 0°C, thioglycollate and benzoic acid. Freezing at 0°C being the simplest of all is recommended for routine use.

For performing the V.D.R.L. test, the reconstituted antigen is to be used during one day.[1] Each ampoule of the V.D.R.L. antigen after reconstitution contains 5 ml amount which is sufficient for testing nearly 200 sera. In most laboratories, however, the number of samples received per day is far less. Thus if the test is performed daily or even on alternate days, a lot of antigen has to be wasted. On the other hand if the laboratory decides to store the sera to collect adequate number before doing the test, the reports are delayed. This problem can be solved if a method can be designed to preserve the reconstituted antigen without diminution of the activity. Earlier attempts at stabilizing the reconstituted antigen by various preservatives met with moderate success. Hintons[2] indicator glycerine could preserve the antigen for three weeks. Rappaport and Eichhorn[3,4] achieved the same for two weeks using ice. Portnoy et al[5] could preserve the antigen containing Choline chloride for one week with 0.01% merthiolate. Bossak and Duncan[6] failed to preserve the antigen with bovine albumin and glycerine but could successfully preserve it with 0.01% benzoic acid for two weeks at 37°C, for four weeks at 23-29°C and for 4-6 weeks at 4°C. The present study deals with a wide variety of physical and chemical methods to evaluate their ability to preserve the reconstituted V.D.R.L. antigen.

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Material and Methods

Antigen was reconstituted in batches of one or two ampoules and then pooled in a flat bottomed bottle. Using this antigen the titres of four pooled batches of sera were assessed. The antigen was divided into nine equal portions. One each was kept at 25°C (room temperature), at 4°C in the refrigerator and 0°C in the freezing chamber. To the rest of the aliquots the following

TABLE 1
Effect of storage, time and various preservatives on the reactivity of VDRL antigen

Method of preservation	Number of days								
	1	3	5	7	9	13	17	22	
Room temperature	LR								Antigen Finished
Refrigerator	SR	SR	SR	SR	SR	SR	SR	SR	
Freezer	SR	SR	SR	SR	SR	SR	SR	SR	
Cysteine	SR	LR							
Merthiolate	SR	SR	SR	SR	SR	SR	SR	SR	
Formaldehyde	LR								
Thioglycollate	SR	SR	SR	SR	SR	SR	SR	SR	
Benzoic acid	SR	SR	SR	SR	SR	SR	SR	SR	

SR — Standard Reactivity LR — Low Reactivity

preservatives were added to obtain the given concentrations:

Cystein	—	0.2%
Merthiolate	—	0.001%
Formaldehyde	—	0.4%
Thioglycollate	—	0.05%
Benzoic acid	—	0.02%
Potassium metabisulfite	—	10 parts per million

All these aliquots were kept in the refrigerator at 4°C. The reproducibility of the results obtained was checked by repeating the entire study twice using 0.05 per cent concentration of cystein and the same that of formaldehyde in addition to the above mentioned concentrations. Each time the different pools of sera were used.

Patients' sera

Each of the four to five pools of known positive sera was inactivated and its antibody titre determined with freshly prepared V.D.R.L. antigen.

The sera were stored at 0°C and their titres were determined with the antigens containing various preservatives at regular intervals. Sera were inactivated at 56°C for 10 minutes before every repeat test.

Results

Table 1 shows the effect of storage, time and various preservatives on the

TABLE 2
Effect of storage time and various preservatives on the reactivity of VDRL antigen

Method of preservation	Number of days												
	1	3	5	7	9	13	17	22	27	32	37	42	47
Room temperature	LR												
Refrigerator	SR	SR	SR	SR	SR	SR	SR	SR	LR				
Freezer	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR
Cysteine (0.05%)	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR		
Cystein II (0.2%)	SR	LR											
Merthiolate	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR		
Formaldehyde (0.05%)	SR	SR	SR	SR	SR	SR	SR	SR	LR				
Formaldehyde (0.4%)	LR												
Thioglycollate	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR
Benzoic acid	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR
Potassium metabisulfite	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	LR		

SR — Standard Reactivity LR — Lower Reactivity

reactivity of the reconstituted V.D.R.L. antigen in study 1. The antigen preserved at 25°C and with 0.4 per cent formaldehyde, even a day later, failed to detect any reactivity with the known positive serum. The antigen to which 0.2% cystein was added showed autoagglutination. The rest of the preserved antigens showed standard reactivity (maximum of one tube difference in titre) till 22nd day by which time all the preserved antigen was exhausted. In view of this the amount of preserved antigens was increased in the second and third study. Since cystein at 0.2% concentration gave autoagglutination and formaldehyde diluted the reactivity of the V.D.R.L. antigen at a concentration of 0.4%, both the reagents were used at 0.05% concentration in the following studies.

In the second and third study the antigens preserved at 4°C and with 0.05% formaldehyde showed standard rectivity for 17 days and 22 days respectively. The antigen kept at 0°C and the antigen to which cystein at a concentration of 0.05%, merthiolate, benzoic acid, thioglycollate and potassium metabisulfite at the given concentrations were added, could be preserved for 27 to 42 days (Table 2).

Discussion

It has been advocated that the V.D.R.L. antigen should be used within the same day after reconstitution[1]. An attempt was made to preserve the same by various physical and chemical methods in this study. Low temperature being one of the factors for the preservation of lipids, was used to maintain the reactivity of the V.D.R L. antigen upto 17 days at 4°C and upto 42 days at 0°C. The various chemicals, most of them being reducing agents like cystein, merthiolate, formaldehyde, thioglycollate, benzoic acid and potassium metabisulfite at given concentrations could also preserve the antigen for 22 days to 42 days. The loss of the activity of the lipids is generally determined by their rate of hydrolysis and oxidation[7]. At lower temperatures and by means various reducing agents, this process of degradation is slowed down to a great extent. Preservation with thioglycollate, benzoic acid and at 0°C are the most efficient methods out of all those tried in this study. Freezing at 0°C being the simplest could be advocated for routine use in the laboratory.

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