Use of plasma instead of serum in laboratory tests for infectious mononucleosis

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SYNOPSIS Until recently, the exclusive use of serum in all laboratory tests for detecting heterophile antibody has remained unquestioned. This communication describes a series of studies which demonstrate that comparable results are obtained when sequestenized plasma is used instead of serum in the conventional differential absorption technique and in two slide screening tests. It is confirmed that significant differences in the final differential absorption titres can result from variations in the agglutinability of erythrocytes from different sheep but such differences when present are the same for plasma as for serum. The advantages of using plasma for routine purposes are briefly discussed.

In the laboratory investigation of infectious mononucleosis suspects, a major inconvenience has not only been the need to obtain both anticoagulated and clotted blood samples for haematological and serological tests respectively but the samples frequently have to be sent to separate departments or even different laboratories for the appropriate examinations. Further disadvantages are that adequate amounts of blood may be difficult to obtain from children, and in those instances where routine haematological examination first raises the suspicion of infectious mononucleosis the diagnosis cannot be confirmed or refuted until a clotted sample is obtained.

Recently Coplan (1968) reported that plasma obtained from sequestenized blood can be satisfactorily used as an alternative to inactivated serum in modifications of both the sheep cell presumptive and differential absorption tests. This paper describes a series of studies undertaken to evaluate and compare the use of sequestenized plasma and serum in the conventional differential absorption technique and two commercially available slide screening tests.

Materials and Methods

Throughout this study paired serum/plasma samples were used. The plasma samples were obtained from whole blood with dipotassium ethylenediamine tetraacetic acid (EDTA, sequestrene) as anticoagulant in a concentration of 1 mg per millilitre of whole blood. Previous observations (unpublished) have shown that both serum and plasma can be stored at −4°C without alteration in the heterophile antibody activity.

DIFFERENTIAL ABSORPTION TEST

Complement is inactivated by heating test serum at 56°C for 30 minutes. Samples, each of 0.25 ml, of the inactivated serum are then added to 0.25 ml of guinea-pig kidney emulsion (a 20% suspension in normal saline with 0.5% phenol as preservative, Oxoid). Absorption is allowed to proceed in both mixtures for one hour at room temperature before centrifuging. To the first of a series of 10 tubes (2 × 0.5), each tube containing 0.25 ml of normal saline, is added 0.25 ml of the supernatant from the guinea-pig kidney-absorbed serum and then doubling dilutions are made. Of a freshly prepared 1% sheep cell suspension 0.25 ml is then added to each tube, thus giving 10 final dilutions ranging from 1:8 to 1:4,096. After mixing thoroughly the test is left standing for 18 hours at room temperature before reading the end point of the titration, which is recorded as the last dilution showing macroscopic agglutination. The same procedure is followed in a second series of tubes except that the serum absorbed with ox cells is used. In this laboratory the lowest titres accepted as being diagnostic are 1:64 before and 1:32 after absorption with guinea-pig kidney emulsion. If lower titres are detected it is routinely
requested that the tests be repeated after an interval of seven to 10 days.

**SLIDE SCREENING TESTS**

The two tests evaluated were (a) the Denco infectious mononucleosis test (Denver Laboratories Ltd) and (b) the Monospot test (Ortho Diagnostics Ltd). Both were carried out according to the instructions supplied by the manufacturers.

**Experiment 1**

A pilot study was undertaken to determine and compare the agglutination titres obtained with serum and plasma in the differential absorption test described using fresh sheep erythrocytes.

Paired samples from 10 patients with proven infectious mononucleosis and 10 healthy adult controls were tested.

The reciprocal pre- and postabsorption titres obtained with serum and plasma from the infectious mononucleosis patients, recorded in Table I, are seen to be more or less identical. No false positive or anomalous reactions were found with the control plasma samples.

<table>
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<tr>
<th>Patient</th>
<th>Serum</th>
<th>Plasma</th>
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<td>J.S.</td>
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<tr>
<td>M.K.</td>
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<tr>
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<td>1,024</td>
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</table>

Table I Comparison of serum and plasma sheep cell agglutinin titres

- TBA: before absorption
- TAAGPK: after absorption with guinea-pig kidney emulsion
- TAAE: after absorption with beef erythrocytes

**Experiment 2**

The effect of varying concentrations of sequestrene was investigated by carrying out differential absorption tests with a single serum and four plasma samples obtained from five proven cases of infectious mononucleosis and five healthy controls. The plasma samples were obtained from whole blood specimens to which sequestrene had been added to give final concentrations of 2, 8, 16, and 32 mg sequestrene/ml whole blood respectively.

In each instance, identical saline and postabsorption titres were given by the serum and the four plasma samples. Thus, sequestrene, even when present in gross excess, does not appear to interfere with the test in any way.

**Experiment 3a**

As the differential agglutination titres of individual sera may vary considerably according to the reactivity of erythrocytes from different sheep (Keiper, 1945; Zarafonetis and Oster, 1950; Lee, Davidson, and Slaby, 1968), serum and plasma from one infectious mononucleosis patient were each tested against erythrocytes from nine different sheep. The latter consisted of fresh cells obtained from six live sheep (S1-S6) of known age, sex, and breed (kindly supplied by the Rowett Institute, Bucksburn) and from three sheep (S7-S9) at the time of their slaughter.

The reciprocal titres obtained before and after absorption with guinea-pig kidney emulsion are recorded in Table II. The results show that with erythrocytes from any one sheep the serum and plasma titres do not vary by more than 1 dilution, thus confirming the findings of experiment 1. In addition the erythrocytes from all nine sheep gave comparable postabsorption titres with the serum and plasma from this one patient.

<table>
<thead>
<tr>
<th>Sheep Cells</th>
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<th>Plasma</th>
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<tbody>
<tr>
<td></td>
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<td>TAAGPK</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>S9</td>
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</tbody>
</table>

Table II Comparison of serum and plasma agglutinin titres obtained with erythrocytes from nine different sheep

*See footnotes to Table I

**Experiment 3b**

Despite the latter finding, a more extensive investigation was undertaken to assess the degree of variation in the final differential absorption titres which could be attributed to differences in the agglutinability of erythrocytes from different sheep and, if found to exist, whether such variation was the same for plasma as for serum.

Paired samples from 20 individual patients with infectious mononucleosis were therefore each tested against erythrocytes from eight different sheep. The
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former were selected to provide a wide spectrum of heterophile-antibody activity (range 1:32-1:4,096); the latter consisted of fresh cells obtained from the same live sheep (S₁-S₄) used in the previous experiment, a pooled sample of these cells (S₅), and a sample of commercially available sheep erythrocytes (S₁₁).

The following results were obtained: (a) the post-absorption titres for any one serum and its corresponding plasma were never found to vary by more than 1 dilution with erythrocytes from any one of the eight sources; (b) six of the eight erythrocyte sets (S₁-S₃, S₅, S₁₀, and S₁₁) gave titres, never differing by more than 1 dilution, with all the serum/plasma pairs; (c) of the two remaining erythrocyte sets, both from live sheep, S₅ gave lower titres (2-3 dilutions) with seven of the serum/plasma pairs while S₉ gave a lower titre (2 dilutions) with one of the pairs.

The results confirm that plasma can be satisfactorily used instead of serum in the differential absorption technique described; significant differences in the final differential absorption titres can result from variations in the agglutinability of erythrocytes from different sheep; and such differences, when present, are the same for plasma as for serum.

Experiment 4

To compare the use of serum and plasma in the Denco and Monospot tests for infectious mononucleosis, 200 paired samples were screened by both methods and a differential absorption test was then performed on all the serum samples.

One hundred samples were obtained from 84 selected patients with active or convalescent infectious mononucleosis providing a range of heterophile antibody activity of 1:8 to 1:4,096. The remaining 100 samples were obtained from 100 individuals suspected of having infectious mononucleosis clinically but whose serum gave a negative reaction with one or both slide tests. None of the latter group had a diagnostic differential absorption test and none had a peripheral blood picture characteristic of infectious mononucleosis.

The following results were obtained: (a) all 200 plasma samples gave results identical to that of the corresponding serum; (b) all serum/plasma pairs from the patients with proven infectious mononucleosis gave positive reactions with both slide tests; (c) 97 of the serum/plasma pairs from the ‘non-infectious mononucleosis’ patients gave negative results with both tests; and (d) of the remaining three, one gave a false positive reaction with the Denco infectious mononucleosis test and two a false positive reaction with the Monospot test, although in both of the latter weak heterophile antibody titres, 1:8 and 1:16, were detected by the differential absorption test.

The results clearly demonstrate that unactivated sequestrenized plasma can be used satisfactorily in both slide techniques and that the tests themselves are of comparable diagnostic accuracy.

Discussion

The exclusive use of serum in the many procedures available for detecting heterophile antibody has continued ever since it was employed in the original sheep cell presumptive (Paul and Bunnell, 1932) and differential absorption (Davidsohn, 1937) tests.

When these techniques were introduced over 30 years ago serum was undoubtedly more convenient to obtain as anticoagulants were then only in limited use and centrifugation of whole blood samples presented an additional problem. As these difficulties no longer exist, it would appear reasonable to question whether the exclusive use of serum should be perpetuated.

I have been unable to trace any report stating why plasma cannot or should not be used yet it is common knowledge that most laboratories will, at some time or other, have used plasma when serum has not been available.

Recently Coplan (1968) has shown that sequestrenized plasma can be satisfactorily used in modifications of both the sheep cell presumptive and differential absorption tests. Further it has been reported that the heating of serum for 30 min at 56°C is not essential for the ‘spot’ test which uses horse erythrocytes preserved in 3·8 % sodium citrate (Lee, Davidsohn, and Pancerzyn, 1968). The latter also indicated that preliminary results with non-inactivated plasma collected with heparin or tri-potassium ethylenediamine tetaacetate (K₃H-Etda) appeared satisfactory.

The evidence presented in the present communication would appear to substantiate these preliminary findings. It is also considered relevant to point out that the first step in the conventional differential absorption technique is to inactivate the test serum by heating for 30 min at 56°C. When plasma is similarly treated, fibrinogen is converted to fibrin at 56°C is its temperature of irreversible precipitation. The small fibrin ‘clot’ can be easily removed and hence the test virtually carried out with ‘serum’.

Provided more extensive investigations confirm that the sensitivity and diagnostic accuracy of the tests are not significantly diminished when sequestrenized plasma is used instead of serum, the use of
the former would have many advantages in the routine diagnosis of infectious mononucleosis. These would include collection of only a single blood sample, simplification of the tests which could be performed more quickly and cheaply, and probably most important of all, haematologists would be in a position to correlate at once the peripheral blood picture with the heterophile antibody status of all infectious mononucleosis suspects.

References


