Serum gentamicin assays of 100 clinical serum samples by a rapid 40°C Klebsiella method compared with overnight plate diffusion and acetyltransferase assays

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SUMMARY We have compared the results of gentamicin assay of 100 clinical serum samples by a rapid 40°C plate diffusion method, an overnight plate assay at 37°C, and radioactive acetyltransferase methods. The results of assay obtained by both plate diffusion methods agreed closely. There was excellent correlation between the results of acetyltransferase and plate assays provided that human serum gentamicin standards were used for the acetyltransferase assay and turbid sera were excluded. Lipaemic sera were associated with falsely high results by the acetyltransferase method. There was no difference in specificity between the methods when antibiotics other than gentamicin were present. Much less skilled technician time was required to perform the rapid 40°C plate method than the radioactive acetyltransferase method. The 40°C plate method is preferred for routine serum gentamicin assays in our clinical laboratories.

Serum gentamicin assays are frequently necessary to ensure adequate dosage and to avoid toxic levels in patients with impaired renal function. Most laboratories in Britain use a plate diffusion technique for gentamicin assay but the results obtained by this method are often highly misleading (Reeves and Bywater, 1975). Recent studies on factors affecting plate assays may help to standardise the plate diffusion method and improve the reliability of the results obtained (Shanson and Daniels, 1975; Shanson and Hince, 1977). When Klebsiella is used as the assay organism plate diffusion assays can be read after 4 to 6 hours as well as after overnight incubation (Reeves, 1972). The Klebsiella strain NCTC 10896 is resistant to many antibiotics and is particularly suitable for gentamicin assay (Waterworth, 1973). The speed of assay with this organism can be increased by raising the temperature of incubation to 40°C and using a heavy inoculum surface seeded on to DST medium; assay results are then available after 2½ hours (Shanson et al., 1976).

A rapid radioactive acetyltransferase method has been recommended recently for gentamicin assay. This method is highly reproducible and very specific (Broughall and Reeves, 1975a and b).

The main purpose of the present study was to compare the results of serum gentamicin assay of 100 consecutive sera from patients receiving gentamicin using the following methods: (1) 2½ hours at 40°C plate diffusion method, (2) 18 hours at 37°C plate diffusion method, (3) radioactive acetyltransferase method using human serum gentamicin standards, and (4) radioactive acetyltransferase method using horse serum gentamicin standards.

The specificity of the four methods was also compared by adding different antibiotics to sera containing gentamicin and then assaying the serum gentamicin by each method.

Material and methods

One hundred serum samples were collected from 40 patients receiving gentamicin. Some of the patients had impaired renal function and many were receiving antibiotics other than gentamicin at the time of assay. Each serum was assayed by the rapid 40°C method and the results were usually communicated to the clinician within 3 hours of collection of the serum sample. Next day the results of overnight plate assay were read and the radioactive acetyltransferase assays were performed.
PLATE DIFFUSION ASSAYS

Oxoid Diagnostic Sensitivity Agar (DST), CM261, was used for assay at pH 7.4. Thirty millilitres agar were poured into 100 mm square plates which were then levelled. Plates were stored for one to four days at room temperature before use. On the day of use the plates were pre-dried for 30 minutes at the temperature of assay.

Aliquots of an overnight peptone water culture of Klebsiella Edwardsii var. atlantae NCTC 10896 were stored at −70°C and a fresh sample was thawed each week. From this the Klebsiella was sub-cultured onto blood agar and the next day several colonies were emulsified into peptone water. A few drops of the peptone water suspension were added to 100 ml nutrient broth and this was incubated at 37°C overnight. Dilutions of culture, were made with 100 or 1000 μg/ml gentamicin. When a high result was expected the test serum was diluted with an equal volume of pooled human normal serum. A previous study showed that the results obtained with this horse serum were the same as those with human serum (Shanson and Daniels, 1975).

Incubation plates were washed three times with tris-hcl, pH 7.4 buffer, and finally in distilled water. The papers were dried under an infrared lamp placed in scintillation fluid and counted in an Intertechnique SL30 liquid scintillation counter.

Each test serum was assayed using the same set of horse serum gentamicin standards that were used for plate diffusion assays and also with a set of human serum gentamicin standards.

PREPARATION OF SERA CONTAINING MIXTURES OF ANTIBIOTICS

A pooled normal human serum sample was prepared containing 3-6 μg/ml gentamicin. This sample was divided into several aliquots, to each of which was added a second antibiotic. The second antibiotic was either carbenicillin, 100 μg/ml, lincomycin, 30 μg/ml, tetracycline, 4 μg/ml, cephradine, 25 μg/ml, cefazolin, 25 μg/ml, or a mixture of trimethoprim, 5 μg/ml, and sulphamethoxazole, 100 μg/ml.

Results

STANDARD CURVES OF PLATE DIFFUSION AND ACETYLTRANSFERASE METHODS

ASSAYS

The data obtained with the plate diffusion method were compared with those obtained with the acetyltransferase assay method for gentamicin standards. The plates were incubated at 40°C for 18 hours and the results were tabulated.

GENTAMICIN ASSAY OF CLINICAL SERUM SAMPLES BY THE DIFFERENT METHODS

The results of gentamicin assay by the four methods are shown in Fig. 2. Good correlation was obtained between the results of the 24-hour plate diffusion method and those of the acetyltransferase method that used human serum gentamicin standards. The results of the 24-hour plate diffusion methods agreed closely with the results of the acetyltransferase assay method.
Serum gentamicin assays of 100 clinical serum samples by a rapid 40°C Klebsiella method

Fig. 1 Comparison of standard curves using human (—) and horse (——) serum gentamicin standards by the acetyltransferase (A) and 18 h plate diffusion (B) methods.

Fig. 2 Comparison of results of 100 clinical serum gentamicin assays by (A) two plate diffusion methods, and (B) two acetyltransferase methods using either human (●) or horse (○) serum gentamicin standards with the 2½ h plate diffusion method.

0.90, slope = 1.25, intercept = 0.20). With eight out of the 100 gentamicin assays of clinical serum samples there was a difference in result of greater than 35% between the 2½ hour plate diffusion assay and the acetyltransferase method using human serum gentamicin standards. Most of these sera were very turbid or haemolysed.

The results of assay of sera which were grossly turbid are shown in Table 1. Overnight plate diffusion assays at 37°C gave results within 0.2 µg/ml of those obtained with the rapid 40°C plate assay. The acetyltransferase method using horse serum standards gave higher results than those observed with the same method using human serum standards. In every instance the highest results were achieved with the acetyltransferase methods. The fluorimetric assay method, performed independently at another hospital, gave results which correlated closest with the plate diffusion method.

Comparison of the antibiotic specificity of the plate diffusion and acetyltransferase assays

Table 2 shows the result of assay of a serum containing gentamicin alone compared with the same serum containing one other antibiotic in addition. Both the plate diffusion and acetyltransferase methods appeared to be specific for gentamicin in the
presence of either carbenicillin, cotrimoxazole, tetracycline, cephradine, cefazolin, or lincomycin in the concentrations stated in the table.

Discussion

The results of clinical serum gentamicin assay by the rapid 40°C plate method agree closely with those of overnight plate assay. Good correlation between these plate diffusion methods has been observed previously in a study using simulated test sera (Shanson et al., 1976).

There was good correlation between the plate diffusion and acetylttranserase methods using human serum gentamicin standards provided that very turbid or haemolysed sera were excluded. All the turbid sera examined in this study were grossly lipaemic and were from two patients. One patient received intralipid intravenously but the other patient had no parenteral lipid therapy. The latter patient was an obese alcoholic patient who was on the intensive care unit. Higher results were obtained with these lipaemic sera by the acetylttranserase assay compared with the plate and fluorimetric assay methods. It has been suggested that lipids coat the phosphocellulose paper used in the acetylttranserase method, making it difficult for any unreacted radio-active labelled C14 acetylcoA (Broughall and Reeves, 1975b) to be washed away completely, and that this method is contraindicated in patients receiving intralipid. No previously published data are available showing how much the results are affected by turbid sera.

Unlike plate diffusion assay the horse serum gentamicin standards gave different standard curves for the human serum standards with the acetylttranserase assay. This resulted in higher values of serum gentamicin from test sera when horse rather than human serum standards were used. Only human serum gentamicin standards can be recommended for the radioactive enzyme assay if the results are expected to correlate closely with those of plate diffusion assay.

Rapid gentamicin assays have the advantage that advice on the next dose of gentamicin can be given on the same day that the serum samples are received. On a Saturday morning and on other days when serum samples are not received until after the middle of the day methods that can give a result in under 3 hours are to be preferred. Most laboratories do not have access to a liquid scintillation counter and the 40°C plate method can then be recommended instead. The radioactive method is particularly suitable for small samples which may be

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### Table 1

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>Assay method</th>
<th>2½ h at 40°C plate diffusion</th>
<th>Radioactive* acetylttranserase</th>
<th>Automated* fluorimetric*</th>
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<tr>
<td>8</td>
<td>2-4</td>
<td>3-0</td>
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<td>4-5</td>
<td>8-4</td>
<td>5-7</td>
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<tr>
<td>Mean and standard deviation</td>
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<td>9-7 ± 4-6</td>
<td>6-2 ± 3-2</td>
<td></td>
</tr>
</tbody>
</table>

*Using same human serum gentamicin standards.
†Independently performed at another hospital.

### Table 2

<table>
<thead>
<tr>
<th>Serum</th>
<th>Plate assay result (mg/l)</th>
<th>Acetylttranserase result</th>
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<tr>
<td></td>
<td>2½ h; 40°C</td>
<td>18 h; 37°C</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l)</td>
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<td>3-6</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l) + Lincomycin, 30 mg/l</td>
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<td>3-2</td>
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<tr>
<td>Gentamicin 'X' (mg/l) + Cefazolin, 25 mg/l</td>
<td>3-4</td>
<td>3-9</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l) + Cephradine, 25 mg/l</td>
<td>3-1</td>
<td>3-6</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l) + Tetracycline, 4 mg/l</td>
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<td>3-3</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l) + Carbenicillin, 100 mg/l</td>
<td>3-0</td>
<td>3-2</td>
</tr>
<tr>
<td>Gentamicin 'X' (mg/l) + Trimethoprim, 5 mg/l, and Sulphamethoxazole, 100 mg/l</td>
<td>3-2</td>
<td>3-3</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3-22 ± 0-20</td>
<td>3-44 ± 0-26</td>
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relevant in neonatal or paediatric practice. The enzyme method can also be quicker as results are available after 1½-2 hours compared with 2½-3 hours for the 40°C plate assay. In practice, a result which is available after 3 hours is usually fast enough. Once the radioactiv enzyme method has been in use for a short time it is not too difficult to achieve reliable results. In contrast, a more experienced and skilled technician is needed to perform reliable plate assays.

The main advantages offered by the 40°C plate assay over the acetyltransferase assay are simplicity, cheapness, safety, and, most important, a considerable saving in skilled technician time. The total amount of skilled technician time required to perform serum gentamicin assays on a couple of serum samples by the acetyltransferase method is about 45 minutes compared with about 5 minutes for the rapid plate assay.

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References


