New Microtechnique for the Leptospiral Microscopic Agglutination Test

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A new microtechnique has been developed for the detection of leptospiral antibodies in serum by the microscopic agglutination test. The test was set up in a microtiter transfer plate held in a transfer plate holder, resting on a transfer plate cover. Live leptospiral antigen was added and a second transfer plate cover was placed over the transfer plate during 2 h of incubation at 32 C. After incubation the bottom cover was removed and the complete unit was placed in a specially designed base plate containing microscope slides (50 by 75 mm). The serum/antigen mixture was ejected on to the microscope slides by means of a sharp tap. The agglutination was then read using a 10× objective, 10× eyepieces, and a dry, dark field condenser.

The microscopic agglutination test is a well proven and accepted test for the detection of leptospiral antibodies in animal and human sera (2, 4).

Until 1965, the test was very time consuming and tedious, and a large amount of live antigen was required. Galton et al. (3) described a microtechnique for the test in which the agglutination was read directly from microtiter plates using a dark ground microscope. The advantages of this method were a 75 to 80% saving in time and an eightfold saving in serum and antigen, but difficulty was reported in cleaning the plates and the optical clarity was impaired by any small scratches in the wells.

In 1973, Cole et al. (1) described a modification of the Galton method in which microtiter plates with flat-bottom wells were used in conjunction with a dark field microscope equipped with a 10× long working distance objective. He considered his method better on the grounds that he could read negative results, whereas Galton et al. (3) could not.

Experienced technologists at this laboratory have found difficulty in obtaining consistent results using the method of Cole et al. (1), probably due to the inadequate optical properties of the styrene plates. Disposable vinyl plates give more consistent results due to the better optical properties. However, the expense of only using these plates once or even a few times is a serious disadvantage.

Carter and Chapman (unpublished data)

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used a transfer plate in a transfer plate holder for preparing the dilutions and then used a Leitz 10× long working distance objective lens to read the agglutination directly from the transfer plate, but this method had limited success. In our experience, the method described in the present paper surpasses all the foregoing techniques in ease of reading and consistency.

MATERIALS AND METHODS

Microtiter equipment. The following equipment was used: transfer plates (Cooke Microtiter, Ltd.); transfer plate holders (Cooke Microtiter, Ltd.); transfer plate lids (Cooke Microtiter, Ltd.); microdroppers calibrated to drop 0.025 ml (Cooke Microtiter, Ltd.); microdiluters calibrated to carry 0.025 ml (Cooke Microtiter, Ltd.); and Gold Seal microscope slides (50 by 75 mm) (Clay Adams). The special base plate (Fig. 1) consisted of a perspex block to hold the transfer plate holder securely and locating strips to support two microscope slides (50 by 75 mm) directly under the transfer plate holes to ensure accurate spotting on to the two slides. The test sera were from cattle and 0.01 M phosphate-buffered saline (pH 7.4) was used as diluent. Antigens were live leptospiral cultures from a commercial source or 4-day cultures prepared in Stuarts medium.

Performance of test. Microdroppers and microdiluters were used to prepare dilutions of the sera under test in transfer plates using phosphate-buffered saline as diluent. This laboratory routinely uses final dilutions of 1/20, 1/200, and 1/2,000.

The transfer plate remained in a plate holder at all times. During dilution and incubation the plate holder was placed in a transfer plate cover for additional safety. Live leptospiral antigen was added to the serum dilutions, mixed, and incubated at 32 C

for 2 h with a second transfer plate cover on top of the plate to prevent evaporation of fluid.

After incubation the bottom cover was removed. The transfer plate holder, plate, and top cover were placed directly onto the special base plate described above. With the top cover in place to protect the operator, the complete unit was raised approx-

mately 3 cm above a flat, firm surface, such as a bench top, and given a sharp tap onto the bench.

This was found to be sufficient to allow small drops to fall through the transfer plate onto the microscope slide as individual drops in the pattern of the transfer plate. The transfer plate holder was then removed and the transfer plate and cover were

FIG. 1. Perspex base plate showing locating positions for transfer plate holder and two microscope slides.

FIG. 2. Transfer plate, plate holder, plate cover, and base plate with two microscope slides in position.

dropped into a hypochlorite solution. The microscope slides were removed from the base plate, care being taken not to tilt the slides and so run the drops together. The slides were then read in the normal way using a microscope equipped with 10 $\times$ objective, 10 $\times$ eyepieces, and a dry, dark field condenser (Fig. 2, 3, and 4).

The above technique was compared with the standard method used in this laboratory which entailed dilutions of serum in a microtiter plate and transfer of the antigen/serum mixture from microtiter plate to microscope slide by means of a pipette for detection of agglutination by dark field microscopy.

FIG. 3. Complete unit ready to emit drops from the transfer plate.

FIG. 4. Base plate showing droplet formation on microscope slides.

RESULTS AND DISCUSSION

No differences were found in the degree of agglutination determined by the new technique compared with the standard transfer method.

Although 48 tests are read on each slide, there was adequate time to read the titers before the drops on the slide dried out. No loss of reagents through the holes in the transfer plate was observed during dilution with the microdiluters or incubation, but it is essential to avoid touching the underside of the holes.

When using automatic pipettes, such as the Cooke minipipetter, to add reagents to the transfer plate, it was found that the delivery nozzle needed to be tilted slightly in order to aim the jet towards the sides of the wells. When the jets were arranged vertically above the holes in the transfer plate, slight leakage occasionally occurred.

This laboratory has found that the method described has many advantages over existing methods and it has been adopted in this laboratory because it combines the ease and clarity of

reading the agglutination by the spotting-out method with speed and safety equalling that of the direct reading methods.

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