JOURNAL OF CLINICAL MICROBIOLOGY, July 1983, p. 206–207
0095-1137/83/070206-02$02.00/0

Vol. 18, No. 1

Rapid Indirect Hemagglutination Test for Serotyping *Pasteurella haemolytica*

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Received 17 January 1983/Accepted 30 March 1983

A modified indirect hemagglutination test which enabled *Pasteurella haemolytica* to be serotyped in 3 h is described. There was 100% correlation with the conventional test for both the 15 prototype strains and the 50 field isolates. It was found that nonviable cultures could also be serotyped.

Pasteurella haemolytica can be subdivided into 15 different serotypes (3) on the basis of the indirect hemagglutination (IHA) test first described by Biberstein et al. (1). Later modifications of this test have included the use of a microtiter method (5) and, recently, the use of glutaraldehyde-fixed ox cells (3). In this paper we describe a modification of the IHA test which enables serotyping to be performed from a single colony in under 3 h.

P. haemolytica strains used in this study were either from our own stock reference cultures or from field cases referred to us for serotyping by the Veterinary Investigation Centres in the United Kingdom. Hyperimmune rabbit sera for serotyping were prepared as described previously (3). Rabbits whose sera did not show an IHA antibody titer of >1/64 after nine injections of killed organisms were given two further intravenous injections of live *P. haemolytica* (10^6 CFU per dose) of the appropriate serotype. This almost invariably boosted the titers beyond 1/64, but when higher titers were not obtained, no further attempts were made to increase the titers by further inoculations. For the rapid IHA (RIHA) test one isolated colony was picked from a blood agar plate with a loop or swab stick and emulsified in 1 ml of phosphate-buffered saline containing 0.3% neutral formaldehyde in plastic 1.5-ml microcentrifuge tubes. The bacterial suspension was heated in a water bath at 56°C for 30 min to release the antigen, 0.1 ml of a 5% suspension of glutaraldehyde-fixed ox cells (4) was added, and the mixture was further incubated at 37°C for 30 min. The sensitized erythrocytes were washed three times with phosphate-buffered saline containing 0.3% neutral formaldehyde in a microcentrifuge. Equal volumes (25 μl) of the sensitized erythrocytes and rabbit antisera to each of the 15 serotypes were dispensed with a calibrated dropper into U-bottomed microtiter plates (Dynatech Laboratories, Inc.). The optimum dilution of the antisera had been determined in previous tests. Agglutination was observed after approximately 1 h at room temperature.

A trial was conducted to compare the results of the RIHA test with those of the conventional test. The 15 prototype strains and the 50 field strains were tested, and 100% correlation between the results of the two methods was obtained. In an attempt to further simplify the test we examined the necessity for heat treatment at 56°C. Titration of heated and unheated samples showed that heating increased the amount of antigen available for binding to erythrocytes. It was also found that live cultures were not necessary. Cultures tested weekly over a 4-week period continued to be typed specifically by the RIHA test even after bacteria were no longer viable.

Frank and Wessman (2) have described a rapid plate agglutination test for serotyping *P. haemolytica*. We have found the rapid plate agglutination test not to be reproducible because of unacceptable cross-reactivity.

The modification of the IHA test described here enables serotyping of *P. haemolytica* cultures to be carried out in 3 h after the receipt of a culture on solid medium. The standard IHA test requires overnight culturing in broth to provide the antigen. The RIHA test allows savings in time and materials, can be carried out on one colony which need not be viable, and should facilitate the examination of cultures when a multiplicity of serotypes is suspected.

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