Improved Hemagglutination Test for Identifying Type A Strains of Pasteurella multocida

G. R. CARTER

Department of Microbiology and Public Health, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48823

Received for publication 12 March 1972

A simple, improved, indirect hemagglutination test is described for the recognition of Type A strains of Pasteurella multocida. It involves the treatment of mucoid cultures with testicular hyaluronidase. Hydrolysis of the capsular hyaluronic acid presumably releases the specific antigen for adsorption to erythrocytes.

An indirect hemagglutination (IHA) procedure was developed for the identification of different capsular antigens of Pasteurella multocida and, on the basis of these differences, four different types or groups, viz., A, B, C, and D were designated (1). An additional type E was identified later (3), and the type C category was subsequently dropped because of difficulties in recognition (4, 8). That strains possessing the same capsular or K antigen could possess different somatic or O antigens was later shown by Namioka and Bruner (7).

Some strains of P. multocida produce capsular hyaluronic acid which can be depolymerized by testicular and bacterial hyaluronidases (2, 5). Carter (2) noted that the presence of capsular hyaluronic acid, particularly on mucoid type A organisms, resulted in strains being untypable by IHA. Perreau et al. (9) confirmed this observation and described a procedure for the routine treatment of mucoid cultures with hyaluronidase. Many strains that were not typable by the conventional IHA procedure were found to be type A after hyaluronidase treatment. The procedure described by Perreau et al. (9) required the treatment of 50 ml of a heavy suspension with hyaluronidase. The method described below is simpler and can be carried out with little modification of the original procedure.

The IHA procedure employed was that described originally (1). Some optional minor modifications are described below. Although blood agar has been used most frequently in the typing procedure, clear agar media such as Tryptose agar (Difco), yeast-peptone-cystine medium (8), and dextrose starch agar (Difco) have the advantage of making possible the recognition of different colonial variants.

The mucoid culture was washed off the plate with 3 ml of 0.1 M phosphate-buffered saline (PBS), pH 6. To the suspension was added 1 ml of PBS, pH 6, containing 15 National Formulary units (50 viscosity-reducing units) of testicular hyaluronidase (Haver-Lockhart Laboratories, Shawnee, Kans.). For convenience, reconstituted hyaluronidase was stored in a frozen state at −20°C. The suspension was placed in a water bath at 37°C for 3 to 4 hr. It was then heated to 56°C for 30 min, after which the bacteria were removed by centrifugation. Adequately treated organisms can be centrifuged at 1,315 × g in 30 min. Group O human erythrocytes were added to the decanted supernatant fluid, and the remainder of the procedure was performed as described earlier (1).

The bacteria remaining after centrifugation and removal of the supernatant fluid may be used in a plate agglutination procedure (G. R. Carter, Vet. Rec., 1972, in press). The results of this procedure provide information on the occurrence of somatic or O antigens.

The original IHA procedure, with the minor modifications described below, was employed. All serum dilutions were made in 0.3% formalinized saline. The addition of 0.1 ml of packed erythrocytes, instead of 0.2 ml, to the culture extract was thought to increase the sensitivity of the test. The final concentration of the erythrocyte suspension was reduced from 1 to 0.5%. To reduce the amount of serum required, 0.05 as compared with 0.08 ml, tests were set up in smaller tubes (Kahn; 12 × 75 mm) in which the final volume of diluted serum plus red-cell suspension was 0.25 in-
stead of 0.4 ml. In addition to the first reading of the tests, after the tubes were placed at room temperature, a second reading was made by agitating the tubes after leaving them at room temperature (25°C) overnight. The latter reading confirmed the earlier results and was easier to read.

A large number of strains have been treated with hyaluronidase prior to performing the IHA test. Typical results obtained with and without hyaluronidase treatment of four freshly isolated mucoid strains are shown in Table 1. Hyaluronidase by itself in the concentration used did not render the erythrocytes agglutinable by the antipasteurella sera after the 3 to 4 hour treatment period. For this reason in later tests heating of the suspension after hyaluronidase treatment was omitted.

Several observations suggest how hyaluronic acid results in strains being untypable by IHA. It was originally thought that its adsorption to red cells blocked adsorption of the specific antigen. However, it was found that amounts of hyaluronic acid as large as 100 μg/ml of a 0.5% suspension of erythrocytes did not block the adsorption of specific antigen. The action of hyaluronidase would seem to involve the release of specific antigen located below or within the capsular hyaluronic acid. Heating of the suspensions at 56°C for 30 min, as described in the original IHA procedure, presumably aids the extraction of the specific antigen but is not sufficient if large amounts of capsular hyaluronic acid are present. In contrast to some lipopolysaccharides, heating is not necessary for the adsorption to erythrocytes of the type A capsular antigen.

**Table 1. Effects of hyaluronidase treatment on results of the indirect hemagglutination test**

| Type A strains | Indirect hemagglutination titers* | Before hyaluronidase | After hyaluronidase |
|----------------|----------------------------------|---------------------|-------------------|
| P1406 (bovine) | 1:20                              | >1:320              |
| P199 (porcine) | 1:20                              | 1:80                |
| P126 (porcine) | Negative                          | >1:320              |
| P161 (porcine) | Negative                          | >1:320              |

* Type A rabbit antiserum.
* Bacteria were heated at 56°C for 30 min as in the original procedure (1).

**LITERATURE CITED**

1. Carter, G. R. 1955. Studies on *Pasteurella multocida*. I. A hemagglutination test for the identification of serological types. Amer. J. Vet. Res. 16:481–484.
2. Carter, G. R. 1956. Some characteristics of type A strains of *Pasteurella multocida*. Brit. Vet. J. 114:356–357.
3. Carter, G. R. 1961. A new serological type of *Pasteurella multocida* from Central Africa. Vet. Rec. 73:1052.
4. Carter, G. R. 1963. Proposed modification of the serological classification of *Pasteurella multocida*. Vet. Rec. 75:1264.
5. Carter, G. R., and E. Annau. 1953. Isolation of capsular polysaccharides from colonial variants of *Pasteurella multocida*. Amer. J. Vet. Res. 14:475–478.
6. Mannheim, W. 1961. Uber die Erkennung mukoider Stamme von *Pasteurella multocida* (P. septica) aufgrund ihrer Hyaluronidase-Sensibilitat und ihre Eigennung zum biologischen Nachweis des Enzmys. Zen-tralbl. Bakteriol. Parasitenk. Infekionskr. Hyg. Abt. Orig. 183:180–195.
7. Namioka, S., and D. W. Bruner. 1963. Serological studies on *Pasteurella multocida*. IV. Type distribution of the organisms on the basis of their capsule and O groups. Cornell Vet. 53:41–53.
8. Namioka, S., and M. Murata. 1961. Serological studies on *Pasteurella multocida*. I. A simplified method for capsule typing of the organism. Cornell Vet. 51:498–507.
9. Perreau, P., A. Vallee, and L. Renault. 1962. Types serologiques de *Pasteurella multocida* isolés chez le porc, en France. Bull. Acad. Vet. Fr. 35:129–134.